# A GENETIC ANALYSIS OF WHITE-MARGINED FLOWERS IN THE JAPANESE MORNING-GLORY

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TABLE OF CONTENTS

| | PAGE |
|---|---|
| INTRODUCTION | 199 |
| The quantitative variation of the white margin | 200 |
| The simplest segregation | 201 |
| The occurrence of complemental factors | 203 |
| The result of cross 26×UN | 203 |
| The result of cross M3×WN | 214 |
| Segregation of three factors concerning the production of white-margined flower | 220 |
| The occurrence of a special factor which varies the quantity of the white margin | 226 |
| The result of cross 324×71–2 | 226 |
| The result of cross 50×71–2 | 235 |
| The result of crosses RA×71–2 and RB×71–2 | 236 |
| SUMMARY | 239 |
| LITERATURE CITED | 241 |

## INTRODUCTION

Some races of the Japanese morning-glory (*Pharbitis Nil*) bear flowers with white peripheral margins[1] on the corollas. Notwithstanding the fact that the white-margined flower is a variation recognized rather recently, it now enjoys great popularity in our gardens. The pattern is commonly transmitted as a simple dominant to the self-colored flower (TAKEZAKI 1916, MIYAZAWA 1918, IMAI 1919, 1925, HAGIWARA 1922a, 1922b). TAKEZAKI (1916) found cases, however, in which the white-margined flower behaves as a recessive to the self-colored condition, owing to the presence of an inhibitor. When such dominant and recessive white margins were concerned in the segregation of $F_2$, it was found that there were 13 self-colored and 3 white-margined flowers. The fact was later confirmed by HAGIWARA (1922b, 1926).

The writer's further study showed the occurrence of complemental factors concerned in the formation of the white-margined flower, besides the presence of the inhibitor above cited. As was already pointed out by IMAI (1919, 1925) and HAGIWARA (1922a, 1926), the common factor for the white-margined flower is linked quite closely with the *contracta* factor,

[1] This condition is called "Fukurin" in Japanese.

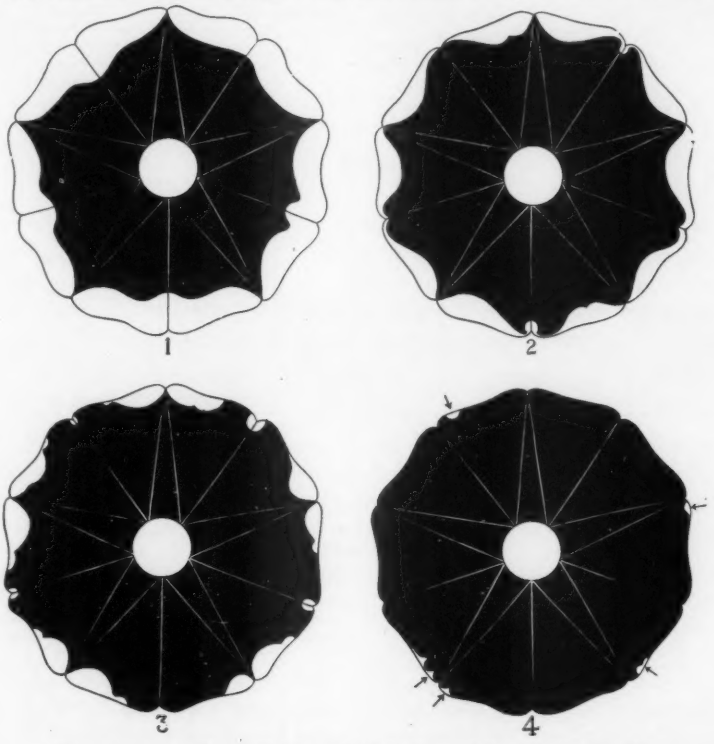
the former being one of the complemental factors. Another complemental factor is that of being linked very closely with the factor for the *Nandina* leaf,[2] which is uncommon in our gardens. The fact that the development of the white margin was somewhat variable according to its environment, under which the plant is raised, was repeatedly shown by these Japanese investigators. Some cases of the writer's experiment, however, revealed themselves to be so extremely variable that they could hardly be considered as ordinary cases, and genetic analysis showed that such conspicuous variations are, at least, due mainly to the segregations of a factor, which reduces the quantity in development of the corolla pattern, and is very susceptible of fluctuation in its manifestations. As a result, an apparently self-colored flower is frequently produced in the hybrid progeny, but it gives rise to white-margined flowers making a large part of the members in the subsequent generation. And further, this factor is moderately linked with the factor *c* (IMAI 1921), which is responsible for the white flower with a colored stem.

## THE QUANTITATIVE VARIATION OF THE WHITE MARGIN

The development of the white margin on the corolla depends somewhat upon the environment under which the plants were grown and upon the time of observation at which the record of the characters was taken. Even in one individual the white margin varies generally in such a manner that the development of pattern, however perfect it may be, is inclined to diminish, as a rule, little by little in later stages of plant growth. MIYAZAWA (1918) observed a case in which the production of the self-colored flower in some $F_2$ families conspicuously exceeded all expectations, and he attributed this result to the dry conditions of his pot culture. The manifestation of the common white-margined flower, which acts as a dominant to the self-colored condition, is due to the occurrence of the factor $F^a$ (see a later section). In the homozygotic white-margined flower the development of the pattern is almost complete, though sometimes it may diminish to some extent, while it becomes invariably imperfect in the heterozygotic flower. According to the writer's experience variation in the development of the corolla pattern is not so conspicuous even in the heterozygotic flower when the plant is raised on open ground and the record is taken in a suitable period from normally grown individuals. But when both complemental factors are segregated or a partial inhibitor is concerned (see the explanation of a later section) we can see a remarkable series of

[2] This peculiar form of the leaf somewhat resembles that of *Nandina domestica*, an evergreen shrub. The genetic data concerning this leaf will be given in another paper.

variations ranging almost continuously from a very meagre pattern to perfection. In the latter case, variation frequently leads to a self-colored flower without its manifestation. Such a flower cannot be distinguished from the ordinary constant type without a breeding test. Thus the quantitative variation of the pattern runs sometimes in a continuous series of




FIGURES 1 to 4. The artificial standards in the variation of white margin.

gradation from completeness to total absence. In order to measure the quantity of the pattern, the writer has, for convenience, classified the pattern into four classes; namely, "complete," "half," "slight" and "dotted" the standard of each degree being given in figures 1 to 4.

## THE SIMPLEST SEGREGATION

The $F_1$ of a cross performed between a white-margined flower and a self-colored one usually represents a white peripheral pattern on the corolla

to the degree of "half" or nearly so. In this case, the simplest segregation takes place generally at the ratio of 3:1. The data representing this simplest segregation of two alternative characters are collected in table 1.

TABLE 1

*$F_2$ data making simplest segregation.*

| CROSS | WHITE-MARGINED | SELF-COLORED | TOTAL | SEGREGATING PERCENT OF RECESSIVE PLANTS |
|---|---|---|---|---|
| α×65 | 67 | 24 | 91 | 26.37 |
| 324×α | 43 | 12 | 55 | 21.82 |
| α×314 | 80 | 22 | 102 | 21.57 |
| 320×A5 | 30 | 17 | 47 | 36.17 |
| 50×M4 | 238 | 88 | 326 | 26.99 |
| 323×316 | 36 | 9 | 45 | 20.00 |
| 323a×316 | 66 | 15 | 81 | 18.52 |
| 220×316 | 243 | 71 | 314 | 22.61 |
| 220×65 | 127 | 36 | 163 | 22.09 |
| 65×220 | 265 | 105 | 370 | 28.38 |
| 224×220 | 30 | 15 | 45 | 33.33 |
| 65×324 | 93 | 30 | 123 | 24.39 |
| Total | 1318 | 444 | 1762 | 25.20 |
| *Expected* | *1321.5* | *440.5* | *1762* | *25.00* |

With some of these $F_2$, the $F_3$ rearing was made, but no new result was found beyond expectation. We shall now tentatively set down the summarized data of $F_3$ plants derived from two crosses in table 2.

TABLE 2

*Summarized data of $F_3$ progenies from two crosses 324×316 and 65×326.*

| CROSS | PHENOTYPE OF $F_2$ AND NEXT GENERATION | | NUMBER OF PEDIGREES | WHITE-MARGINED | SELF-COLORED | TOTAL | SEGREGATING PERCENT OF RECESSIVE PLANTS |
|---|---|---|---|---|---|---|---|
| 324×316 | White-margined | breeding true | 19 | 669 | | 669 | 0.00 |
| | | segregating | 33 | 622 | 213 | 835 | 25.51 |
| | Self-colored | breeding true | 17 | | 428 | 428 | 100.00 |
| 65×326 | White-margined | breeding true | 27 | 683 | | 683 | 0.00 |
| | | segregating | 33 | 795 | 266 | 1061 | 25.07 |
| | Self-colored | breeding true | 13 | | 389 | 389 | 100.00 |
| Total | White-margined | breeding true | 46 | 1352 | | 1352 | 0.00 |
| | | segregating | 66 | 1417 | 479 | 1896 | 25.26 |
| | Self-colored | breeding true | 30 | | 817 | 817 | 100.00 |

Upon comparison of genotypes of white-margined $F_2$ flowers, after impartially omitting those pedigrees which contained only a few individuals

(below ten in number), we found the result of table 3, in which the number observed quite satisfies the expected ratio of 1:2.

TABLE 3

*Comparison of genotypes of white-margined $F_2$ flowers.*

| CROSS | NUMBER OF HOMOZYGOTIC PEDIGREE | NUMBER OF HETEROZYGOTIC PEDIGREE | TOTAL |
|---|---|---|---|
| 324×316 | 12 | 29 | 41 |
| 65×326 | 20 | 29 | 49 |
| Total | 32 | 58 | 90 |
| *Expected* | *30* | *60* | *90* |

Two crosses given in table 1, $\alpha \times 314$ and $\alpha \times 65$, were composed of segregating numbers just in accordance with the 1:2:1 ratio of "complete" margined flowers, imperfect ones varying from "slight" to "half," and self-colored, as indicated in table 4. We have no accurate knowledge as to how such a result was obtained particularly in these crosses, but it may be

TABLE 4

*$F_2$ result of two crosses segregating into a 1:2:1 ratio.*

| CROSS | "COMPLETE"-MARGINED | "HALF"-MARGINED | "SLIGHT"-MARGINED | SELF-COLORED | TOTAL |
|---|---|---|---|---|---|
| $\alpha \times 314$ | 20 | 42 | 18 | 22 | 102 |
| $\alpha \times 65$ | 27 | 40 | | 24 | 91 |
| Total | 47 | 100 | | 46 | 193 |
| *Expected* | *48.25* | *96 50* | | *48.25* | *193* |

due to the fact that the record was taken in a favorable time to note the genotype in its manifestation. The individuals in the hybrid progeny may have varied during the time of flowering, so it is a matter of a little difficulty to obtain the record of each member at a favorable period on a somewhat larger scaled experiment.

## THE OCCURRENCE OF COMPLEMENTAL FACTORS

### *The result of cross 26×UN*

Since the complemental factors were discovered by BATESON and PUNNETT in a classical experiment, quite a number of similar instances have been reported. The writer also detected such a case of manifestation

in the white-margined flower of the Japanese morning-glory, in the hybrid progeny of a cross 26×UN, the former bearing flowers with "perfect" white margins, while the latter had self-colored corollas. The $F_1$ plants obtained by this cross bore white-margined flowers to the degree of "slight" on an average, and they segregated numerically into 276 white-margined and 159 self-colored individuals, or 1.74:1 in ratio, in the subsequent generation. The investigation of the cause, by which such a phenotypic ratio is developed, makes it advisable to consider the segregation of the other characters at the same time, because the white-margined and the self-colored flowers represented special relationship to *contracta and Nandina* leaf in their segregation. The actual segregation of the $F_2$ result is shown in table 5.

TABLE 5

*$F_2$ data of cross 26×UN representing a complicated segregation.*

| PEDIGREE | NORMAL | | | | | *Nandina* | | | | | TOTALS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-Colored | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | |
| 1 | 15 | 12 | 32 | 5 | 1 | | | | 1 | 14 | 80 |
| 2 | 9 | 12 | 18 | 1 | | | | | | 8 | 48 |
| 3 | 3 | 14 | 18 | 6 | | | | | 2 | 12 | 55 |
| 4 | 10 | 20 | 32 | 2 | 2 | | | | 1 | 18 | 85 |
| 5 | 5 | 15 | 41 | 8 | 2 | | | | | 18 | 89 |
| Total | 42 | 73 | 141 | 22 | 5 | 0 | 0 | 0 | 4 | 70 | 357 |
| | 278 | | | | 5 | 0 | | | 74 | | 357 |

| PEDIGREE | *Contracta* | | | | | Contracted *Nandina* | | | | | TOTALS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-Colored | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | |
| 1 | | | 1 | | 26 | | | | | 4 | 31 |
| 2 | | | | | 8 | | | | | 3 | 11 |
| 3 | | | | | 8 | | | | | 3 | 11 |
| 4 | | | 1 | | 13 | | | | | 5 | 19 |
| 5 | | | 2 | | 13 | | | | | 1 | 16 |
| Total | 0 | 0 | 4 | 0 | 68 | 0 | 0 | 0 | 0 | 16 | 88 |
| | 4 | | | | 68 | 0 | | | 16 | | 88 |
| Grand Total | | | | | | | | | | | 445 |

TABLE 6

*$F_2$ data of cross 26×UN.*

| Pedigree number | NORMAL | | | | | *Nandina* | | | | | *Contracta* | | | | | CONTRACTED *Nandina* | | | | | Total | SUBSERIES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Complete" morphid | "Half" morphid | "High" morphid | "Dotted" morphid | Self-Colored | "Complete" morphid | "Half" morphid | "High" morphid | "Dotted" morphid | Self-colored | "Complete" morphid | "Half" morphid | "High" morphid | "Dotted" morphid | Self-colored | "Complete" morphid | "Half" morphid | "High" morphid | "Dotted" morphid | Self-colored | | |
| 32 | **14*** | 12 | | | | | | | | | | | | | | | | | | | 26 | |
| 34 | 21 | | | | | | | | | | | | | | | | | | | | 21 | |
| 39 | 27 | **0** | | | | | | | | | | | | | | | | | | | 27 | A |
| 40 | 24 | 5 | | | | | | | | | | | | | | | | | | | 29 | |
| 44 | 23 | 11 | | | | | | | | | | | | | | | | | | | 34 | |
| 68 | 13 | **0** | | | | | | | | | | | | | | | | | | | 13 | |
| 5 | 15 | 19 | 4 | | | | | | 1 | 8 | | | | | | | | | | | 47 | |
| 7 | 5 | 7 | 5 | | | | | | | 4 | | | | | | | | | | | 21 | |
| 8 | 18 | 2 | | | | | | | 2 | 3 | | | | | | | | | | | 25 | |
| 13 | 26 | 6 | 6 | | | | | | 4 | 4 | | | | | | | | | | | 46 | |
| 16 | 16 | 2 | 3 | | | | | | 5 | 4 | | | | | | | | | | | 30 | |
| 21 | 26 | 7 | 1 | | | | | | 5 | 12 | | | | | | | | | | | 51 | |
| 22 | 20 | 1 | | | | | | | 4 | | | | | | | | | | | | 25 | B |
| 29 | 26 | 4 | 1 | | | | | | 1 | 6 | | | | | | | | | | | 38 | |
| 47 | 20 | 8 | 2 | | 1 | | | | 2 | 4 | | | | | | | | | | | 37 | |
| 56 | 13 | 5 | 1 | | | | | | 2 | 6 | | | | | | | | | | | 27 | |
| 59 | 47 | 11 | 2 | | 1 | | | | 3 | 10 | | | | | | | | | | | 74 | |
| 62 | 27 | 12 | 1 | | | | | | 12 | 4 | | | | | | | | | | | 56 | |
| 63 | 73 | 9 | | | | | | | 3 | 13 | | | | | | | | | | | 98 | |
| 65 | 18 | 20 | 7 | | 1 | | | | 4 | 6 | | | | | | | | | | | 56 | |
| 14 | 5 | 4 | 10 | 2 | 7 | | | | | 9 | | | | | | | | | | | 37 | |
| 49 | 1 | 1 | 4 | 3 | 3 | | | | | 4 | | | | | | | | | | | 16 | C |
| 58 | 8 | 17 | 6 | 2 | 7 | | | | 6 | 13 | | | | | | | | | | | 59 | |
| 3 | 13 | 8 | 1 | | | | | | | | | | | | 4 | | | | | | 26 | |
| 20 | 4 | 10 | 17 | | 1 | | | | | | | 1 | | | 3 | | | | | | 36 | |
| 27 | 4 | 2 | 8 | | | | | | | | | | | | 2 | | | | | | 16 | D |
| 28 | 4 | 1 | 2 | | | | | | | | | | | | 2 | | | | | | 9 | |
| 38 | 4 | 7 | 7 | | | | | | | | | | | | 3 | | | | | | 21 | |
| 71 | 5 | 10 | 3 | | | | | | | | | | | | 7 | | | | | | 25 | |
| 1 | 8 | 13 | 12 | | 1 | | | | 3 | 8 | | | | | 7 | | | | | 4 | 56 | |
| 9 | 1 | 7 | 19 | 3 | 2 | | | | | 4 | | | | | 9 | | | | | 3 | 48 | |
| 10 | 14 | 17 | 33 | 1 | 1 | | | | 7 | 17 | | | 1 | | 16 | | | | | 1 | 108 | |
| 12 | 4 | 6 | 12 | | | | | | 1 | 11 | | | | | 5 | | | | | 4 | 43 | |
| 15 | 3 | 6 | 6 | | | | | | 2 | 2 | | | | | 2 | | | | | | 21 | |
| 17 | 10 | 14 | 46 | 4 | 1 | | | | 2 | 18 | | | | | 16 | | | | | 7 | 118 | |
| 30 | 6 | 12 | 8 | | 1 | | | | 1 | 3 | | | | | 5 | | | | | 1 | 37 | E |
| 31 | 11 | 11 | 24 | 3 | 1 | | | | 1 | 9 | | | 1 | | 13 | | | | | 2 | 76 | |
| 36 | 1 | 6 | 30 | 10 | | | | | 1 | 21 | | | | | 8 | | | | | 5 | 82 | |
| 45 | 4 | 4 | 6 | 2 | | | | | 2 | 3 | | | | | 10 | | | | | | 31 | |
| 46 | 15 | 15 | 18 | | | | | | 2 | 15 | | | | | 6 | | | | | 2 | 73 | |
| 50 | 6 | 17 | 23 | 2 | 1 | | | | | 14 | | | | | 10 | | | | | | 73 | |
| 52 | 2 | 3 | 13 | 4 | | | | | | 13 | | | | | 8 | | | | | 1 | 44 | |
| 53 | 15 | 11 | 8 | | 2 | | | | 4 | 6 | | | | | 10 | | | | | 3 | 59 | |
| 64 | 11 | 16 | 14 | 3 | | | | | | 14 | | | | | 14 | | | | | 2 | 74 | |
| 67 | 6 | 12 | 15 | 3 | 1 | | | | 1 | 11 | | | 1 | | 16 | | | | | 1 | 67 | |
| 11 | | | | | | | | | 8 | 6 | | | | | | | | | | | 14 | |
| 43 | | | | | | | | | | 2 | | | | | | | | | | | 2 | F |
| 48 | | | | | | | | | | 6 | | | | | | | | | | | 6 | |
| 54 | | | | | | | | | | 5 | | | | | | | | | | | 5 | |
| 18 | | | | | | | | | 2 | 7 | | | | | | | | | | 4 | 13 | |
| 24 | | | | | | | | | 6 | 7 | | | | | | | | | | 2 | 15 | |
| 51 | | | | | | | | | 4 | 10 | | | | | | | | | | 3 | 17 | G |
| 60 | | | | | | | | | | 5 | | | | | | | | | | 1 | 6 | |
| 61 | | | | | | | | | 6 | 14 | | | | | | | | | | 5 | 25 | |
| 66 | | | | | | | | | 4 | 3 | | | | | | | | | | 4 | 11 | |
| Total of 7 pedigrees | | | | | | | | | | | | | | | 132 | | | | | | 132 | H |
| Total of 9 pedigrees | | | | | | | | | | | | | | | 148 | | | | | 40 | 188 | I |
| Total of 2 pedigrees | | | | | | | | | | | | | | | | | | | | 12 | 12 | J |

* Bold face figures indicate the character of the respective parental $F_2$.

The fact that the factor for *contracta* links with that for the white-margined flower was pointed out by IMAI (1919, 1925) and HAGIWARA (1922a, 1926), the frequency of crossovers being only about 1 percent. If the same factor of the white-margined flower is concerned in segregation in the present cross, there should naturally be found some data indicating a special relation to the segregations of the margined condition and of *contracta*. The $F_2$ results observed, as was shown in table 5, were complicated by simultaneous segregation of another factor of the white-margined flower, so the exact nature of the segregation cannot clearly be realized. But it can be roughly said from the results obtained that the majority of the segregating *contracta* bloomed into self-colored flowers, while a large part of the normal, with the exception of the normal *Nandina*, bore flowers with white margins. Concrete evidence, therefore, may be sought in the $F_3$ results shown in table 6. In an actual trial, 6 pedigrees in class D of table 6 were segregated in a dihybrid fashion in regard to the factors for *contracta* and the white-margined flower. The segregation of these pedigrees are regarded to be the same in nature, so by adding them together a total sum is obtained as shown in table 7. The segregating numbers of the normal and *contracta* are 120 and 22 respectively, indicating a some-

TABLE 7

*Summarized data of 6 pedigrees in class D of table 6.*

| | NORMAL | | | | | *Contracta* | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | |
| Total observed | 34 | 38 | 38 | 0 | 1 | 0 | 1 | 0 | 0 | 21 | 133 |
| | 110 | | | | 1 | 1 | | | | 21 | |
| *Expected* | *99.095* | | | | *0.655* | *0.655* | | | | *32.595* | 133 |

$\chi^2 = 5.676$ $P = 0.131$

what larger discrepancy in the ratio (deviation $= \pm 13.5$, while probable error $= \pm 3.48$). Being probably affected by such deviation, the value of goodness of fit in table 7 was still no more than 0.131. This consideration makes an explanation necessary for in the present cross we are concerned also with the segregation of the factor for the white-margined flower linked with that of *contracta* at about 1 percent of the crossover frequency. But with the evidence of this special relation only we cannot understand the whole aspect of the $F_2$ segregation. Upon inspecting the items in the

normal *Nandina* of table 5, we are struck by the fact that they all bear self-colored flowers, excepting a few "dotted"-margined flowers, somewhat similar to the case of *contracta*. Fourteen pedigrees in class B of table 6 are considered to make dihybrid segregation of the factors for the white-margined flower and *Nandina*. Table 8 contains the summarized data of the numbers thus observed. Many plants with *Nandina* leaves bore self-

TABLE 8

*Summarized data of 14 pedigrees in class B of table 6.*

| | NORMAL | | | | | *Nandina* | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | |
| Total observed | 350 | 112 | 34 | 0 | 3 | 0 | 0 | 0 | 48 | 84 | 631 |
| | 496 | | | | 3 | 0 | | | 132 | | |
| *Expected* | *471.75* | | | | *1.5* | *1.5* | | | *156.25* | | *631* |

$\chi^2=8.010$ P=0.046

colored flowers, including a considerable number of "dotted"-margined flowers. From classes F and G of table 6 we may conclude that there exists a close relation between the manifestations of the "dotted" margin and full coloration in the normal *Nandina*. The "dotted" margin frequently fails to develop in different flowers of a plant which is recorded as having that characteristic. As the result of this fluctuation the record may contain some errors, and for this reason, we cannot attach much importance upon the observed numbers of the "dotted"-margined and self-colored flowers in the normal *Nandina* leaves. In accounting for the appearance of "dotted"-margined flowers in the normal *Nandina* type, attention may be called to the probable occurrence of a modifier, which affects the development of the white margin to a slight degree. Whether this is the case or not, we may be permitted to recognize such a "dotted"-margined flower to be a modified form in the self-colored condition, and so this form may be added to the self-colored flowers of the normal *Nandina*, when consideration is made in regard to the segregating aspect of the factor for the white-margined flower, which holds a close relation to that of *contracta*. Thus we can take the quantitative estimation in intensity of the linkage from 496 normal leaves with white-margined flowers, 3 normal leaves with self-colored flowers, no *Nandina* leaves with white-margined flowers and 132 *Nandina* leaves with self-colored flowers (the last including

"dotted"-margined flowers from the allowance above-designated) as indicated in table 8. The gametic ratio estimated from these is 209.03:1, the crossovers being 0.48 percent or nearly 0.5 percent in frequency. Here the value of P, as indicated in table 8, is not so high as desirable, due mainly to the influence of a discrepancy observed in the segregation of the normal *versus Nandina* allelomorphs. From the segregating numbers of 273 normals, 74 *Nandinas*, 72 *contractas* and 16 contracted *Nandinas* (total 435), it can be recognized that the factors concerned in the present data segregated freely, without linkage ($\chi^2=9.713$, $P=0.022$).[3] Consequently it may be clear that the factor, which governs the formation of the white margin on the corolla and links with that for *contracta*, differs from that which is linked with the factor for *Nandina*, indicating the occurrence of two working factors for the manifestation of the white margin. As a result, there was no simple segregation of the corolla pattern in $F_2$ of the present cross. Almost all members that were found in the classes of *Nandina*, *contracta* and contracted *Nandina* of table 5 (with actually only 4 exceptions among 162 individuals), had self-colored flowers, or they consisted of those regarded practically as self-colored flowers in their factor relationship, so that the total obtained by adding the observed numbers of these three classes corresponds to seven-sixteenths of the total $F_2$. Then the ratio of white-margined and self-colored flowers is expected to be 9:7, indicating the occurrence of two complemental factors for the formation of the corolla pattern. In practice, however, those plants carrying a recessive foliage factor or factors, were somewhat weak in their growth, and they gave rise to some discrepancy in the ratio of segregation; the actual deformity in number of the self-colored flowers may not be regarded to be of any serious source in factor analysis. In an attempt to realize the genetic relationship of the present cross, on such a basis, it will be necessary to establish the following factors:

1. $F^a$, $f^a$—The $F^a$ factor concerns the formation of the white margin on the corolla, and it holds 1 percent of the crossovers with the factor for *contracta*.

2. $F^b$, $f^b$—In the coexistence of $F^a$, the factor $F^b$ completes the manifestation of the white margin. Neither factor, acting by itself, produces the trait, but both work in complemental cooperation. The factor $f^b$ is linked closely with that for *Nandina*. In the combination of $F^a$ and $f^b$ in general, no white margin is produced on the corolla, but when they enter into *Nandina* leaves, they often manifest themselves in "dotted"-margined flowers.

[3] The value of P is low, but we cannot attribute this, as far as the data show, to the result of any factorial complexity beyond the recognition of a superficial deviation.

Now we shall attempt to settle the genetic composition of the parents of the present cross. In addition to the complemental factors above-represented, it may be necessary to give those for *contracta* (*d*) and *Nandina* leaf ($n_n$) in this consideration. The genetic composition of 26 is considered to be a quadruple dominant from the fact that the specimen is a normal-leafed *non-contracta* with white-margined flowers. Then its partner, UN, is regarded as one carrying a quadruple recessive constitution. In the cross of such a parentage, the $F_1$ should be heterozygous for

TABLE 9

*Theoretical expectation in $F_2$ of cross 26×UN.*

| CHARACTER | RATIOS | |
|---|---|---|
| | Calculated on the basis of such gametic ratios: Between *d* and $f^a$, x:1 Between $n_n$ and $f^b$, y:1 | Applied such real figures: x=100 y=200 |
| Normal with white-margined flower | $9x^2y^2+12x^2y+12xy^2+16xy+6x^2+8x+6y^2+8y+4$ | 3672622404 (or 45565) |
| Normal with self-colored flower | $6x^2y+6xy^2+20xy+3x^2+10x+3y^2+10y+5$ | 36553005 (or 454) |
| *Nandina* with white-margined flower | $6x^2y+8xy+3x^2+4x+4y+2$ | 12191202 (or 151) |
| *Nandina* with self-colored flower | $3x^2y^2+6xy^2+4xy+2x+3y^2+2y+1$ | 1224200601 (or 15188) |
| *Contracta* with white-margined flower | $6xy^2+8xy+4x+3y^2+4y+2$ | 24281202 (or 301) |
| *Contracta* with self-colored flower | $3x^2y^2+6x^2y+4xy+3x^2+2x+2y+1$ | 1212110601 (or 15038) |
| Contracted *Nandina* with white-margined flower | $4xy+2x+2y+1$ | 80601 (or 1) |
| Contracted *Nandina* with self-colored flower | $x^2y^2+2x^2y+2xy^2+x^2+y^2$ | 412050000 (or 5112) |
| Total | $16x^2y^2+32x^2y+32xy^2+64xy+16x^2+32x+16y^2+32y+16$ | 6626089616 (or 81810) |

four sets of allelomorphs, and produce the following sorts of gametes: (Here the gametic ratio in the linkage of *d* and $f^a$ is denoted by x:1 (x>1), and that of $n_n$ and $f^b$ by y:1 (y>1)).

$$xyDN_nF^aF^b+xDN_nF^af^b+yDN_nf^aF^b+xDn_nF^aF^b+ydN_nF^aF^b+1DN_nf^af^b+xyDn_nF^af^b+1dN_nF^af^b+1Dn_nf^aF^b+xydN_nf^aF^b+1dn_nF^aF^b+yDn_nf^af^b+xdN_nf^af^b+ydn_nF^af^b+xdn_nf^aF^b+xydn_nf^af^b$$

As formerly worked out, the numerical relations are about x=100 and

$y=200$, so if we apply these actual figures the above formula will be changed into:

$$20000DN_nF^aF^b+100DN_nF^af^b+200DN_nf^aF^b+100Dn_nF^aF^b$$
$$+200dN_nF^aF^b+1DN_nf^af^b+20000Dn_nF^af^b+1dN_nF^af^b+1Dn_nf^aF^b$$
$$+20000dN_nf^aF^b+1dn_nF^aF^b+200Dn_nf^af^b+100dN_nf^af^b+200dn_nF^af^b$$
$$+100dn_nf^aF^b+20000dn_nf^af^b$$

On fertilization with free combinations of these 16 different gametes, the $F_2$ members may be produced at an unusual ratio as indicated in table 9. If a calculation is made with this ratio as a basis, the theoretical numbers applied to the actual $F_2$ total will be as shown in table 10.

TABLE 10

*Comparison of observed and expected numbers in $F_2$ of cross 26×UN.*

| | NORMAL | | *Nandina* | | *Contracta* | | CONTRACTED *Nandina* | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| | White-margined | Self-colored | White-margined | Self-colored | White-margined | Self-colored | White-margined | Self-colored | |
| Observed | 268 | 5 | 0 | 74* | 4 | 68 | 0 | 16 | 435 |
| *Expected* | *242.278* | *2.414* | *0.803* | *80.758* | *1.600* | *79.960* | *0.005* | *27.182* | *435* |

$\chi^2=16.843$ $P=0.019$

* This includes "dotted"-margined flowers.

The value of P is very low, but we can hardly attribute this to any genetic speciality, because the discrepancy may be regarded mainly as having been influenced by a deficiency of the recessive classes, as formerly stated.

Next we may compare the actual $F_3$ results with our theoretical expectations. Class A of table 6 contains the pedigrees which bred true to the normal and white-margined flowers, so it includes those having the constitution of $DDN_nN_nF^aF^aF^bF^b$. The fact that these families carry both the dominant factors for the white margin in a double dose, gives reason for the expectation of all flowers to be "complete"-margined in their progeny. But the practical result was that they often contained a number of the "half"-margined flowers. This, however, is probably due to a mere fluctuation. The parental plants of Nos. 39 and 68 were recorded to be "half" in the development of its white margin on the corolla, but all offspring of these pedigrees grew up to bear "complete"-margined flowers. Number 40 showed almost the same result. These data may endorse the fact that the homozygotic white pattern sometimes expresses fluctuation to such an extent. Class B contains the pedigrees segregated

in the factor for *Nandina* and one of the factors for the formation of white-margined flowers, as formerly cited, their genetic composition consequently being considered as $DDN_nn_nF^aF^aF^bf^b$. In this factor composition a linked assortment should occur in the subsequent generation. Class C includes three pedigrees. The individuals with white-margined flowers (from normal) and those with self-colored corollas (total of those from the normal and of all *Nandina*, the latter containing specimens with "dotted"-margined flowers) being 63 and 49, respectively, in the total of this class, the result coincided perfectly with the segregating ratio of 9:7. From this ratio the pedigrees are regarded to have segregated into two complemental factors. Here segregation took place in the *Nandina* allelomorphs, but bred true to the normal habit (*non-contracta*). Consequently their genetic constitution may be $DDN_nn_nF^af^aF^bf^b$. This is the only class containing the pedigrees produced by crossover gametes+non-crossover gametes, as far as the trial breeding tests were concerned; namely, they are the result of $DN_nF^af^b+Dn_nf^aF^b$ or $Dn_nf^af^b+DN_nF^aF^b$, the former in each union being a crossover gamete, while the latter is a non-crossover gamete. In table 11, the observed total of these three pedigrees and the expectation are given in comparison. Here the expected numbers of the normals with white-margined and with self-colored flowers, *Nandina* with white-margined and with self-colored flowers are calculated from a ratio of 362406:122406:1203:160401, respectively.

TABLE 11

*Summarized data of 3 pedigrees in class C of table 6.*

| | NORMAL | | | | | *Nandina* | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted" margined | Self-colored | "Complete"-margined | "Half"-margined | "Slight"-margined | "Dotted"-margined | Self-colored | |
| Total observed | 14 | 22 | 20 | 7 | 17 | 0 | 0 | 0 | 6 | 26 | |
| | 63 | | | | 17 | 0 | | | 32 | | 112 |
| *Expected* | *62.792* | | | | *21.208* | *0.208* | | | *27.792* | | 112 |

$\chi^2=2.250$ $P=0.527$

The value of P is 0.527, indicating about one occurrence with such deviation in every two trials. The pedigrees assembled in class D are considered to be $DdN_nN_nF^aF^aF^bf^b$, as their segregation in the $F_2$ generation took place in both allelomorphic sets of $F^b$, $f^b$ and $D$, $d$. Class E, however, contains the pedigrees making tetrahybrid segregation as $F_2$ did.

As to the segregation of the corolla pattern in this class, we count, in total, 612 white-margined flowers (the sum of those plants found in the normal and *contracta*) and 398 self-colored flowers (the sum of those having "dotted"-margined flowers and self-colored ones of *Nandina*, and all individuals with self-colored flowers found in *contracta* and contracted *Nandina*); the result having considerably deviated from the theoretical numbers of 568 and 442 calculated from the ratio of 9:7 (here deviation = $\pm 43.875$, probable error = $\pm 10.63$), though in the main we may accept the occurrence of the ratio in this case. Both recessive *Nandina* and *contracta* individuals were fewer in number than expected, and especially the discrepancy of the ratio in the segregation of the latter was evident. These properties of deviation may have been employed somewhat remarkably, in obtaining such a departure in the segregating numbers with respect to the corolla pattern, as a close association of the characteristics took place. Table 12 shows an application of the theoretical numbers to the observed ones of those 16 pedigrees.

TABLE 12

*Summarized data of 16 pedigrees in class E of table 6.*

| | NORMAL | | *Nandina* | | *Contracta* | | Contracted *Nandina* | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| | White-margined | Self-colored | White-margined | Self-colored | White-margined | Self-colored | White-margined | Self-colored | |
| Total observed | 609 | 11 | 0 | 196 | 3 | 155 | 0 | 36 | 1010 |
| *Expected* | *562.531* | *5.605* | *1.864* | *187.506* | *3.716* | *185.654* | *0.012* | *63.111* | *1009.999* |

$\chi^2 = 28.340$ $P = 0.0002$

The goodness of fit is remarkably low, but here we cannot make any consideration for further steps than the recognition of such a superficial cause as above-described.

The foregoing statements were concerned with the examination of the offspring of normal $F_2$. In attempting a further discussion on the results of *Nandina* $F_2$, we may ascertain that class F can be considered as $DDn_nn_nF^aF^af^bf^b$ from its results, and class G as $Ddn_nn_nF^af^af^bf^b$. In regard to *contracta* $F_2$, we presume the genetic compositions to be $ddN_nN_nf^af^aF^bF^b$ and $ddN_nn_nf^af^aF^bf^b$ for classes H and I, respectively. Lastly, the pedigrees of contracted *Nandina*, of which the data were gathered in class J, are considered to be propagated in quadruple recessive homozygotes.

Tracing back the $F_2$ individuals from the $F_3$ data, an attempt can be

made to analyze the nature of the origin of the gametes, whether produced by crossover mechanism or not, which made up the $F_2$ plants after fertilization. The classes from F to J are all self-colored flowers produced by the lack of at least one dominant factor of the two complemental ones necessary for the production of the white-margined pattern, so it is impossible to determine precisely what genotypes they are in respect to the factors for this character. For this reason, it seems advisable to omit these from our consideration in making the following statement with the data furnished by the classes from A to E. As the first consideration, it will be necessary to try to demonstrate the relation between the allelomorphic set, *d* and $f^a$. Classes A and B being double dominant heterozygotes in these factors, the parental plants of 20 pedigrees of these classes are noted as being produced by the union of two non-crossover gametes, and the same statement will hold good with the origin of 22 pedigrees collected in classes D and E, because these pedigrees produced coupling segregations. The genetic composition of the three pedigrees tabulated in class C, however, being regarded as $DdF^af^a$, their development should start from the union of $DF^a$, a non-crossover gamete, and $Df^a$, a crossover one. Summing up these considerations we have:

| | |
|---|---|
| Non-crossover gametes × non-crossover gametes | 42 pedigrees |
| Crossover gametes × non-crossover gametes | 3 pedigrees |
| Total | 45 pedigrees |

To produce 45 parental plants of these $F_3$ pedigrees, the gametes needed for their development will be duplicated with the total of 45. Of these pedigrees, however, 3 being derived, each from the union of a non-crossover gamete and a crossover one, the items of the above total are:

| | |
|---|---|
| Non-crossover gametes | 87 |
| Crossover gametes | 3 |
| Total | 90 |

In this calculation, the frequency of crossovers is 3.33 percent, practically within 1 percent of the expected value. The same treatment is made as to the relationship between $n_n$ and $f^b$ allelomorphic set, but no crossover gametes could be detected.

A word may be added here in respect to the variation in degree of the white margin. The cases in which "dotted"-margined flowers appeared among the normals in the hybrid progeny were confined to the families

segregated into both complemental factors of white-margined flowers. Thus the $F_2$ families and classes C and E of $F_3$ contained a considerable number of "dotted"-margined flowers. They all segregated as dihybrid, as to the corolla pattern (see class E of table 6). From this, it is quite certain that the "dotted"-margined flower of the normal carries the factors of the white margin in a double heterozygous condition. The parental plants of the pedigrees collected in classes C and E carry double heterozygotic constitution for the corolla pattern, but their white margins were "slight" in degree, with a few exceptions of those having "half"-margined and "dotted"-margined flowers. So we can safely say that the plants double-heterozygotic for the factors of the corolla pattern have the "slight" margins, which, however, vary perceptibly at times into its neighboring degree; that is, from the "dotted" margin to the "half" one. In class E, 11 normal individuals of the self-colored flower were counted, the number somewhat exceeding the expected. It cannot be denied that these self-colored flowers contain some false individuals, carrying double heterozygotic constitution for the corolla pattern, as the result of an extreme fluctuation in their manifestation. We had no case in our trial breeding experiments of normals bearing self-colored flowers, so the evidence to prove this occurrence is still wanting; but such possibility cannot be completely rejected, as far as the writer's experience is concerned. If the case should actually occur, the variation of the white margin in the double heterozygotic plants will be extended practically to the degree of absence or self-colored flower, one of the furthest extremities of the quantitative variation of this pattern.

Now we proceed to discuss the problem as to what degree of development in the corolla pattern corresponds to the flowers single-heterozygotic to the factor $F^a$ or $F^b$. In regard to the effect of the factor $F^a$, we stated already in the former section that its single heterozygotic expression is "half" or "slight." The parental $F_2$ plants of class D in table 6 carry a constitution of $F^af^aF^bF^b$ for the corolla pattern and they had practically their corresponding degree of white margin. The factor $F^b$, however, does not appreciably reduce the amount of manifestation of the corolla pattern in its single heterozygotic state. This evidence may be accepted by looking over the contents of class B in table 6. In most cases the parental plants of these pedigrees had practically the white pattern in a "complete" degree, but they contained some "half" ones. As stated before, the factor $f^b$ working with $F^a$ frequently produces "dotted"-margined flowers in *Nandina* plants. In this, we have an opportunity of seeing what difference there may be in the proportion of the manifestation of the "dotted"

pattern if they are homozygotic or heterozygotic as to the factor $F^a$. The results contained in classes B, C, E, F and G will give something in such a comparative study. Of these, classes B and F are composed of pedigrees homozygotic to $F^a$, while the rest, C, E and G, are heterozygotic to it. The total of the former *Nandina* plants consisted of 35.22 percent "dotted"-patterned flowers, but in the latter *Nandina* only 18.58 percent were "dotted." In this calculation, we cannot neglect the interference of fluctuating variation, which, although slight in quantity, may to some extent affect the values so that it will be almost impossible to rely absolutely on them. The difference between these two values, however, is too much to be regarded as the result of a simple variation; namely, the former value amounts to nearly twice the latter. So we conclude that the chance of the appearance of "dotted"-margined flowers in *Nandina* is considerably large in the homozygotes for the factor $F^a$, when compared with its heterozygotes. In summing up the results, the modes of manifestation of various genotypes in regard to the factors for corolla marking are (table 13):

TABLE 13

*The corresponding manifestations of quantitative development in the corolla pattern to respective genotypes.*

| GENOTYPE | PRESENCE AND ABSENCE OF WHITE MARGIN AND ITS DEGREE | |
|---|---|---|
| | In normal | In *Nandina* |
| $F^aF^aF^bF^b$ | "Complete" | white-margined (degree not clear) |
| $F^af^aF^bF^b$ | "Half"—"slight" | |
| $F^aF^aF^bf^b$ | "Complete" | |
| $F^af^aF^bf^b$ | "Half"—"slight"—"dotted" | |
| $f^af^aF^bF^b$ | Absent | Self-colored* |
| $f^af^aF^bf^b$ | " | " " |
| $F^aF^af^bf^b$ | " | "Dotted"—absent |
| $F^af^af^bf^b$ | " | " † |
| $f^af^af^bf^b$ | " | Absent |

* See discussion in next section.

† The proportion of appearance of "dotted"-margined flower should be less than that of the above.

It must be understood that the table represents only an outline of the various manifestations of the pattern factors under different combinations.

### *The result of cross M3×WN*

Another case in which $F_2$ made dihybrid segregation by the occurrence of two complemental factors for the white-margined corolla, was observed in the hybrid progeny of M3, normal (*non-contracta*), bearing "Rangiku"

leaves[4] and blue flowers with a complete white margin on the corolla, and WN, normal, bearing *Nandina* leaves and white flowers with a colored tube. The $F_1$ plants grew up to the ***non-contracta*** bearing normal, three-lobed leaves and blooming colored flowers with a "slight" white margin. They gave rise to a population of $F_2$ as will be seen in table 14.

TABLE 14

*$F_2$ data of cross M3×WN.*

| PEDIGREE | NORMAL | | | *Nandina* | | | TOTAL |
|---|---|---|---|---|---|---|---|
| | White margined | Self-colored | White Flower | White margined | Self-colored* | White Flower | |
| 1 | 53 | 15 | 23 | 0 | 22 | 5 | 118 |
| 2 | 29 | 9 | 15 | 0 | 9 | 3 | 65 |
| Total | 82 | 24 | 38 | 0 | 31 | 8 | 183 |
| *Expected* | *76.948* | *25.989* | *34.313* | *0.255* | *34.058* | *11.438* | *183.01* |

$\chi^2=2.443$ $P=0.783$

* This contains "dotted"-margined flowers.

Since this paper is chiefly to represent the data concerning the segregation of both corolla pattern and *Nandina*, the results in regard to that of other characters are omitted for brevity. And as the field record of white corolla pattern contained some points to be dissatisfied with the classification of degree, they are put together under "white"-margined without being classified, as usual. The white flowers, however, are taken in the segregating number, because we cannot make direct observation on these flowers, as to whether they have white margin or not.

The individuals having a white-margined flower and a self-colored one counted 82 and 55 respectively, besides 46 individuals of white flowers. The white flower behaves as a simple recessive to the colored condition, due to the segregation of the *R,r* allelomorphs, of which the writer has made a genetic statement elsewhere (IMAI 1921). If the factors for white-margined flowers and that for white flowers are freely assorted—the case is practically true as will be stated later—we can analyze the factors concerned in the present case by omitting the actual number of the white flower segregants. The observed numbers above described of 82 white-margined flowers and 55 self-colored ones correspond almost to the expected numbers of 77.06 and 59.94, respectively, calculated on the basic ratio of 9:7. So here we have also the segregation of two complemental

[4] "Rangiku" is an irregular leaf form having sharp pointed lobes, and transmitted as a recessive to the normal.

factors for the corolla pattern as already met with in the former cross. The present cross, however, is somewhat different, as far as we can see, from the result of the former in this respect that, two parents being both homozygotic to the normal habit, we had no segregation concerning the *contracta*. As a result of such a factor relation, one of the factors for the corolla pattern has no linked factor, and so it is expected to segregate freely in the hybrid progeny of this cross. Thus the allelomorphs concerned may be considered to be four sets: $N_n$:$n_n$, $R$:$r$, $F^a$:$f^a$ and $F^b$:$f^b$. The genetic constitution of M3, one of the parents in this cross, then, must be a quadruple dominant homozygote, $N_nN_nRRF^aF^aF^bF^b$, which is a normal-leafed one bearing colored flowers with a "complete" white margin. Its partner, WN, however, is regarded as a quadruple recessive homozygote, because it is a *Nandina*-leafed one with white flowers, and because the segregation of the corolla pattern occurred in a dihybrid scheme in this cross. In the $F_2$ segregation, however, the actual result was somewhat complicated by the occurrence of linkage between the factors, $n_n$ and $f^b$. Thus the theoretical numbers of the $F_2$ segregation are calculated in table 15.

TABLE 15

*Theoretical ratio of $F_2$ in cross M3×WN.*

| CHARACTER | FORMULA | THEORETICAL RATIO |
|---|---|---|
| Normal with white-margined flower | $27y^2+36y+18$ | 1087218 |
| Normal with self-colored flower | $9y^2+36y+18$ | 367218 |
| Normal with white flower | $12y^2+24y+12$ | 484812 |
| *Nandina* with white-margined flower | $18y+9$ | 3609 |
| *Nandina* with self-colored flower | $12y^2+6y+3$ | 481203 |
| *Nandina* with white flower | $4y^2+8y+4$ | 161604 |
| Total | $64y^2+128y+64$ | 2585664 |

The application of the above ratio to the actual total is represented at the bottom of table 14, showing a high rate of possibility of about eight for every ten trials.

In looking over the $F_3$ table (table 16), we see a perfect agreement, in the main points, between expectation and observation. We will pass over the explanation as to how our factor hypothesis holds good in each pedigree, but give some discussions on several remarkable points, which may need a statement.

1. The factor *r* assorts itself freely from the *Nandina* factor and both complemental factors for the corolla pattern. This evidence can be seen from the segregating contents of the $F_2$ offspring and the $F_3$ classes of

TABLE 16

*$F_2$ data of cross M3×WN.*

| CHARACTER OF PARENTAL $F_1$ | REFERENCE | GENETIC CONSTITUTION | PROGENY NUMBER | NORMAL | | | | | | *Nandina* | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | "Complete"-marginal | "Half"-marginal | "Slight"-marginal | "Dotted"-marginal | Self-colored | White Flower | "Complete"-marginal | "Half"-marginal | "Slight"-marginal | "Dotted"-marginal | Self-colored | White Flower | |
| Normal with white-margined flower | A | $N?R?F^a f^a F^b?$ | 35 | | 1 | | | 1 | | | | | | | | 2 |
| | B | $NNRrF^aF^af^bf^b$ | 4 | 1 | 11 | 4 | | | 4 | | | | | | | 20 |
| | C | $NNRrF^a f^a F^b f^b$ | 12 | 1 | 2 | 7 | 2 | 6 | 3 | | | | | | | 21 |
| | | | 17 | 2 | 2 | 8 | | 2 | 5 | | | | | | | 19 |
| | D | $NnRRF^aF^af^bf^b$ | 5 | 14 | 22 | 3 | 1 | | | | | | 2 | 5 | | 47 |
| | E | $NnRRF^a f^a F^b f^b$ | 8 | 1 | 2 | 14 | 10 | 16 | | | | | 1 | 16 | | 60 |
| | | | 16 | 2 | 2 | 8 | | 6 | | | | | 2 | 3 | | 23 |
| | | | 36 | | 5 | 9 | 1 | 4 | | | | | 1 | 6 | | 26 |
| | F | $NnRrF^aF^af^bf^b$ | 1 | 1 | 10 | 17 | 1 | | 10 | | | | 4 | 1 | 2 | 46 |
| | | | 19 | 1 | 12 | 7 | | | 11 | | | | 7 | 4 | 1 | 43 |
| | | | 30 | | 6 | 7 | | | 11 | | | | | 8 | 1 | 33 |
| | G | $NnRrF^a f^a F^b f^b$ | 3 | 1 | 7 | 14 | 7 | 12 | 6 | | | | 4 | 9 | 4 | 64 |
| | | | 6 | 3 | 5 | 8 | 2 | 5 | 10 | | | | 3 | 5 | 2 | 43 |
| | | | 14 | | | 4 | | 4 | 4 | | | | | 1 | | 13 |
| | | | 34 | 1 | 7 | 11 | | 2 | 7 | | | | 3 | 2 | 2 | 35 |
| Normal with self-colored flower | H | $NNRr f^a f^a (F^bF^b)$ | total of 2 pedigrees | | | | | 46 | 16 | | | | | | | 62 |
| | I | $NnRR f^a f^a (F^b f^b)$ | 9 | | | | | 11 | | | | | | 2 | | 13 |
| | J | $NnRr f^a f^a (F^b f^b)$ | total of 6 pedigrees | | | | | 81 | 31 | | | | | 30 | 6 | 148 |
| *Nandina* with self-colored flower | K | $nnRrF^a?$ $(f^bf^b)$ | 18 | | | | | | | | | | 5 | 10 | 5 | 20 |
| | | | 22 | | | | | | | | | | 4 | 19 | 5 | 28 |
| | | | 24 | | | | | | | | | | 1 | 5 | 2 | 8 |
| Normal with white flower | L | $NNrr?$ $(F^bF^b)$ | total of 3 pedigrees | | | | | | 35 | | | | | | | 35 |
| | M | $Nnrr?$ $(F^bf^b)$ | total of 5 pedigrees | | | | | | 97 | | | | | | 30 | 127 |
| *Nandina* with white flower | N | $nnrr?$ $(f^bf^b)$ | total of 2 pedigrees | | | | | | | | | | | | 15 | 15 |

C, F, G and J. There may be found considerable deviation in the segregating numbers in some cases, probably to be attributed to a superficial disturbance.

2. It is interesting that all flowers of the *Nandina*-leafed individuals, in the classes I and J of the $F_3$ pedigrees, had self-colored corollas, not a single plant revealing itself to bear "dotted"-margined flowers. From this evidence it may be said that the factor $F^b$ cannot manifest a "dotted" margin on its corollas of *Nandina* individuals when its complemental factor is recessively homozygotic. The absence of the corolla pattern on these flowers indicates a conspicious difference in nature between the factors, $F^a$ and $F^b$, because the former frequently produces a "dotted"-margined flower in the $f^bf^b$ plants as was already shown. In class K, we cannot make an accurate calculation of the behaviors of the $F^a$, $f^a$ allelomorphs, directly from these data, but the appearance of "dotted"-margined flowers will tell, at least, that the pedigrees of this class are either homozygotic to the factor $F^a$ or its heterozygotes, though the factor $f^b$ is in a double dose. From the fact that the factor $f^b$ links very closely with the factor $n_n$, one may deduce that these *Nandina* pedigrees naturally contain the linked recessive factor in a homozygous condition as the cross was a coupling case.

TABLE 17

*Total data of linked dihybrid segregation of $n_n$ and $f^b$, contained in this paper.*

| SOURCE | NORMAL WITH WHITE-MARGINED FLOWER | NORMAL WITH SELF-COLORED FLOWER | *Nandina* WITH WHITE-MARGINED FLOWER | *Nandina* WITH SELF-COLORED FLOWER | TOTAL |
|---|---|---|---|---|---|
| 26×UN-$F_3$(class B) | 496 | 3 | 0 | 132 | 631 |
| M3×WN-$F_3$(class D) | 40 | 0 | 0 | 7 | 47 |
| -$F_3$(class F) | 62 | 0 | 0 | 24 | 86 |
| Total | 598 | 3 | 0 | 163 | 764 |
| *Expected* | *571.5* | *1.5* | *1.5* | *189.5* | *764* |

$\chi^2=7.935$ $P=0.048$

3. The pedigrees under class D made a dihybrid segregation in regard to the factors for *Nandina* and white-marginedness, and the segregation occurred in high linkage. In this connection, the dependent assortment shows that it was the allelomorphs of $F^bf^b$ that were concerned with the segregation of the flower pattern in the present case. Class F also made a similar segregation as far as we understood the appearance of white-flowered segregants. If these data are added to class B, table 6, of the cross 26×UN, we should have the total results of linked dihybrid segregation of $n_n$ and $f^b$, as indicated in table 17. From

these segregating numbers the frequency of crossovers is calculated to be 0.39 percent.

4. As already described, the development of the white margin on the corolla differs in a degree in a single heterozygotic condition, according as it rests on $f^a$ or $f^b$. This is also the case in the present cross. The white margin in this cross, however, is weaker in development than in the previous cross, the margin generally becoming considerably smaller in quantity, probably due to the fact that the record was taken somewhat late. As a result of this condition, the impression in the difference of manifestation between the two cases above cited is practically weakened when compared with that of the cross, 26×UN, but we must recognize this underlying fact. Table 18 may serve to make this matter plainer, for in it the actual degree and value of the white margin, in every genotype, are given.

TABLE 18

*Quantitative evidence of the difference in manifestation of single heterozygote between factors $f^a$ and $f^b$ in cross M3×WN.*

| REFERENCE | GENOTYPE O PARENTAL $F_1$ | CLASS | NUMBER OF $F_2$ INDIVIDUAL | AVERAGE PATTERN VALUE* | GENOTYPE OF SEGREGATING WHITE-MARGINED FLOWER AND ITS RATIO |
|---|---|---|---|---|---|
| (1) | $F^aF^af^bf^b$ | B | 16 | 2.81 | all $F^aF^af^bf^b$ |
| (2) | $F^af^aF^bf^b$ | C | 24 | 2.33 | $1F^aF^af^bf^b+2F^af^aF^bf^b$ |
| (3) | $F^aF^af^bf^b$ | D & F | 106 | 2.78 | $1F^aF^af^bf^b+2F^aF^af^bf^b$ |
| (4) | $F^af^aF^bf^b$ | E & G | 124 | 2.19 | $1F^aF^af^bf^b+2F^af^aF^bf^b+2F^aF^af^bf^b+4F^af^aF^bf^b$ |

* The value is calculated on the following basis:

| Degree | Value |
|---|---|
| "Dotted"-margined flower | 1 |
| "Slight"-margined flower | 2 |
| "Half"-margined flower | 3 |
| "Complete"-margined flower | 4 |

The value of double homozygote (1) is expected to be 4, while its actual value is only 2.81. Ref. 3 is single heterozygotic to the factor $f^b$, so it must contain two-thirds of the plants carrying $F^aF^aF^bf^b$ among the white-margined flowers. Practically, however, no considerable diminution in degree is recognizable, because the factor $f^b$ in a heterozygotic condition does not apparently affect the development of the white margin, as stated in a former page. Actually, the average value of the $F_3$ progeny of such plants was 2.78, which is very close to (1). The value of (2), on the contrary, ought to be somewhat lower, when compared with those of (1) and (3), for it contains the factor $f^a$ in a heterozygotic state. The actual

value of (2) was 2.33, and thus confirmed our expectation. The factor $f^b$ does not diminish the development of white margin in a marked quantity in the $F^aF^a$ composition, but somewhat in the $F^af^a$ constitution. The value of (4), therefore, is a step lower, in comparison with the other three classes, as (4) carries double-heterozygotic factors. Actually 2.19 was the average value of (4), and was the lowest development.

5. Considerable difference observed in the frequency of occurrence of "dotted"-margined flowers in the *Nandina* individuals between the pedigrees of homozygotes for the factor $F^a$ and of its heterozygote has been dealt with in the results of the former cross. In the present cross, this conclusion also holds good throughout the data obtained. The frequencies are 41.94 percent and 25 percent in the homozygotes and heterozygotes, respectively. The former is nearly twice as many as the latter, the quantitative relation being almost the same as that observed in the previous cross. From these figures, we can ascertain that about twice as many "dotted"-margined flowers are produced, when both the factors, $F^a$ and $f^b$ are homozygotic, compared with the $F^af^af^bf^b$ in *Nandina* plants. If the factor $f^a$ is homozygous, the plant, however, will remain invariably as bearing self-colored flowers, whatever the constitution of its complemental allelomorphs, $F^b$:$f^b$, may be.

6. Owing to the occurrence of close linkage between the factors $n_n$ and $f^b$ the arrangement of the genotypic ratios of $F_2$ must be considerably modified. In an attempt to inspect the items in regard to the gametes which contributed to the formation of $F_2$ zygotes, we obtained a result as follows: As far as the breeding shows, 14 individuals (classes below H were omitted from the calculation for the reason that in these we cannot accurately detect the behavior of the allelomorphs, $F^b$:$f^b$, without making a test breeding), which were used in the $F_3$ trial, are all the products of the union of non-crossover gametes. If the number thus tested in the previous cross, 26×UN, is added to this, we have 59 in total, and still all of them are the zygotes of non-crossover gametes+non-crossover gametes. The frequency of crossovers between the factors $n_n$ and $f^b$ being considered to be at about 0.4 percent, which tells us the expectation that one plant derived from the union of non-crossover and crossover gametes is obtainable in testing about 125 $F_2$. So it will be natural to have no zygote having such an origin in the test of such a small number as 59 against 125.

7. The genetic ratio of the white-margined $F_2$ is expected to be

$1F^aF^aF^bF^b:2F^af^aF^bF^b:2F^aF^aF^bf^b:4F^af^aF^bf^b$. The data are shown in table 19 together with those of 26×UN.

TABLE 19

*Genotypic comparison of white-margined $F_2$ flowers of crosses 26×UN and M3×WN.*

| GENOTYPE | 26×UN | M3×WN | TOTAL* | EXPECTED | RATIO |
|---|---|---|---|---|---|
| $F^aF^aF^bF^b$ | 6 | 1 | 7 | 6.444 | 1 |
| $F^af^aF^bF^b$ | 5 | 2 | 7 | 12.889 | 2 |
| $F^aF^aF^bf^b$ | 14 | 4 | 18 | 12.889 | 2 |
| $F^af^aF^bf^b$ | 19 | 7 | 26 | 26.778 | 4 |
| Total | 44 | 14 | 58 | 58.000 | 9 |

$\chi^2=4.767$ $P=0.193$

* The pedigrees containing below ten individuals are not counted.

The value of P is so high that one case takes place in every five trials.

## SEGREGATION OF THREE FACTORS CONCERNING THE PRODUCTION OF WHITE-MARGINED FLOWER

The $F_1$ plants obtained by the cross of 500, a pedigree of green-stemmed white flowers producing white seeds, and 65, a pedigree flowering with a purple corolla and "complete" margin, bore colored flowers, but, contrary to expectation, they lacked the corolla pattern. In $F_2$ we found a small number of white-margined flowers among self-colored offspring, besides

TABLE 20

*$F_2$ data of cross 500×65.*

| PEDIGREE | "COMPLETE"-MARGINED | "HALF"-MARGINED | "SLIGHT"- AND "DOTTED"-MARGINED | SELF-COLORED | WHITE FLOWER | TOTAL | WHITE-MARGINED/SELF-COLORED |
|---|---|---|---|---|---|---|---|
| 1 | 3 | 3 | 9 | 66 | 25 | 106 | 0.23 |
| 2 | 1 | 1 | 2 | 26 | 15 | 45 | 0.15 |
| 3 | 1 | 2 | 5 | 76 | 26 | 110 | 0.11 |
| 4 | | 2 | 6 | 25 | 8 | 41 | 0.32 |
| 5 | 2 | 6 | 13 | 153 | 65 | 239 | 0.14 |
| 6 | 1 | 2 | 5 | 61 | 29 | 98 | 0.13 |
| 7 | | 2 | 5 | 94 | 32 | 133 | 0.07 |
| 8 | 2 | 2 | 13 | 117 | 47 | 181 | 0.15 |
| Total | 10 | 20 | 58 | 618 | 247 | 953 | 0.14 |
| | | 78 | | | | | |
| *Expected* | *11.17* | *89.34* | | *614.24* | *238.25* | *953* | *0.16* |

$\chi^2=1.909$ $P=0.593$

the segregants of white flowers. Marginal white of the $F_2$ flowers varied in degree from "dotted" to "complete," as represented in table 20.

The proportion in number of white-margined flowers in the total data of $F_2$ is 0.14:1, as is shown at the bottom of the table, or, in other words, the latter goes over seven times as many as the former in number. These data admit of no hasty analysis. By the progress of the experiment, however, it is revealed that three factors contributed to bring about these results; namely, two complemental factors, as detected in the previous experiment, for the formation of the corolla pattern and an inhibitor for the production of the characteristic. The third factor, as studied by TAKEZAKI (1916) and HAGIWARA (1922b, 1926), entirely suppresses the manifestation of the complemental factors, resulting in a self-colored flower which cannot be distinguished from an ordinary recessive one. We shall designate this factor by a symbol of $F^h$. In the $f^hf^h$ constitution, however, the effect of the complemental factors is visible. The genetic constitution of 65, therefore, can be readily considered to be $F^aF^aF^bF^bf^hf^h$ from the fact that it bears "complete"-margined flowers. To give self-colored flowers in $F_1$, and follow a trihybrid segregation in the subsequent generation, its partner, 500, must be $f^af^af^bf^bF^hF^h$ in constitution. The latter parent being practically a white flower, the present cross thus also

TABLE 21

*Theoretical $F_2$ ratio expected in the segregation of three white-margin factors.*

| GENETIC COMPOSITION | ITS RATIO | CHARACTER | ITS RATIO |
|---|---|---|---|
| $XF^aF^bF^f$ | 81 | self-colored flower | 165 |
| $X f^aF^bF^f$ | 27 | | |
| $XF^a f^bF^f$ | 27 | | |
| $X f^a f^bF^f$ | 9 | | |
| $X f^aF^b f^f$ | 9 | | |
| $XF^a f^b f^f$ | 9 | | |
| $X f^a f^b f^f$ | 3 | | |
| $XF^aF^b f^f$ | 27 | white-margined flower | 27 |
| $xF^aF^bF^f$ | 27 | white flower | 64 |
| $x f^aF^bF^f$ | 9 | | |
| $xF^a f^bF^f$ | 9 | | |
| $xF^aF^b f^f$ | 9 | | |
| $x f^a f^bF^f$ | 3 | | |
| $x f^aF^b f^f$ | 3 | | |
| $xF^a f^b f^f$ | 3 | | |
| $x f^a f^b f^f$ | 1 | | |
| Total | 256 | | 256 |

concerns the segregation of allelomorphs of colored flowers producing black seeds (strictly speaking, these should be considered as "colored seeds" against white ones) *versus* white flowers producing white seeds. Now we shall represent this allelomorphic set by $X$ and $x$. The actual segregation in $F_2$, therefore, took place in these four pairs of factors, wherein the result indicated in table 21 is expected. For brevity, the genetic composition in this table is represented in a simplex form, instead of the duplex indication. Then the ratio in number of the self-colored, white-margined and white flowers is 165:27:64. Taking the former two into

TABLE 22

*Genotypes of white-margined $F_2$ flowers and expectation on their behavior in the next generation.*

| CHARACTER OF $F_2$ | GENOTYPE | ITS RATIO | TOTAL | RESULT OF $F_3$ |
|---|---|---|---|---|
| Self-colored flower | $F^aF^aF^bF^bF^lF^l$ | 1 | 37 | breed true to self-colored |
| | $F^af^aF^bF^bF^lF^l$ | 2 | | |
| | $F^aF^aF^bf^bF^lF^l$ | 2 | | |
| | $F^af^aF^bf^bF^lF^l$ | 4 | | |
| | $F^aF^af^bf^bF^lF^l$ | 1 | | |
| | $F^af^af^bf^bF^lF^l$ | 2 | | |
| | $F^aF^af^bf^bF^lf^l$ | 2 | | |
| | $F^af^af^bf^bF^lf^l$ | 4 | | |
| | $f^af^aF^bF^bF^lF^l$ | 1 | | |
| | $f^af^aF^bf^bF^lF^l$ | 2 | | |
| | $f^af^aF^bF^bF^lf^l$ | 2 | | |
| | $f^af^aF^bf^bF^lf^l$ | 4 | | |
| | $f^af^af^bf^bF^lF^l$ | 1 | | |
| | $f^af^af^bf^bF^lf^l$ | 2 | | |
| | $f^af^aF^bF^bf^lf^l$ | 1 | | |
| | $f^af^aF^bf^bf^lf^l$ | 2 | | |
| | $F^aF^af^bf^bf^lf^l$ | 1 | | |
| | $F^af^af^bf^bf^lf^l$ | 2 | | |
| | $f^af^af^bf^bf^lf^l$ | 1 | | |
| | $F^aF^af^bF^bF^lf^l$ | 2 | 2 | segregate into 3 self-colored and 1 white-margined |
| | $F^af^aF^bF^bF^lf^l$ | 4 | 8 | segregate into 13 self-colored and 3 white-margined |
| | $F^aF^aF^bf^bF^lf^l$ | 4 | | |
| | $F^af^aF^bf^bF^lf^l$ | 8 | 8 | segregate into 55 self-colored and 9 white-margined |
| White-margined flower | $F^bF^aF^bF^bf^lf^l$ | 1 | 1 | breeds true to white-margined |
| | $F^af^aF^bF^bf^lf^l$ | 2 | 4 | segregate into 3 white-margined and 1 self-colored |
| | $F^aF^aF^bf^bf^lf^l$ | 2 | | |
| | $F^af^aF^bf^bf^lf^l$ | 4 | 4 | segregate into 9 white-margined and 7 self-colored |

consideration, the proportion is 1:0.16, very close to the actual ratio of 1:014. Next we shall make the statements by neglecting the segregation of white flowers. The expected ratio of self-colored and white-margined flowers in $F_2$ is 55:9, their $F_2$ genotypes and hereditary behavior in the $F_3$ progeny being indicated in table 22. The $F_3$ table (table 23) contains the results of 19 white-margined and 78 self-colored $F_2$ flowers, the total being

TABLE 23

*$F_3$ data of cross 500 × 65.*

| PEDIGREE NUMBER | "COMPLETE" MARGINED | "HALF" MARGINED | "SLIGHT" MARGINED | "DOTTED" MARGINED | SELF-COLORED | WHITE FLOWER | TOTAL | WHITE MARGINED/ SELF-COLORED |
|---|---|---|---|---|---|---|---|---|
| Total of 14 pedigrees | | | | | 643* | | 643 | 0.00 |
| Total of 38 pedigrees | | | | | 1216 | 343 | 1559 | 0.00 |
| 15 | | | | 2 | 21 | | 23 | 0.10 |
| 28 | 1 | 1 | 3 | 1 | 48 | | 54 | 0.13 |
| 50 | 3 | 1 | 8 | 11 | 102 | | 125 | 0.23 |
| 59 | | | 1 | 1 | 5 | | 7 | 0.40 |
| 67 | | | | 1 | 3 | | 4 | 0.33 |
| 72 | | | | 2 | 6 | | 8 | 0.33 |
| 75 | | 1 | 1 | 2 | 7 | | 11 | 0.57 |
| 77 | | 1 | 2 | 1 | 23 | | 27 | 0.17 |
| 83 | 3 | 2 | 17 | 8 | 54 | | 84 | 0.56 |
| 85 | | | 4 | 6 | 20 | | 30 | 0.50 |
| 3 | | 1 | 1 | 1 | 19 | 9 | 31 | 0.16 |
| 5 | 1 | 3 | 1 | 7 | 30 | 4 | 46 | 0.40 |
| 13 | 3 | 2 | 3 | 9 | 45 | 23 | 85 | 0.38 |
| 14 | | | 1 | 5 | 31 | 17 | 54 | 0.19 |
| 19 | | 1 | 1 | 7 | 60 | 30 | 99 | 0.15 |
| 29 | | 4 | 2 | 3 | 41 | 14 | 64 | 0.22 |
| 47 | 2 | 2 | 2 | 4 | 48 | 19 | 77 | 0.21 |
| 51 | | | | 3 | 12 | 3 | 18 | 0.25 |
| 60 | 1 | | 1 | 2 | 15 | 3 | 22 | 0.27 |
| 69 | 2 | 5 | 7 | 6 | 31 | 13 | 64 | 0.65 |
| 71 | | | | 1 | 9 | 2 | 12 | 0.11 |
| 73 | | | | 1 | 6 | 1 | 8 | 0.17 |
| 81 | | 1 | 1 | 2 | 11 | 2 | 17 | 0.36 |
| 82 | | 1 | 4 | 3 | 39 | 10 | 57 | 0.21 |
| 92 | 1 | 3 | 6 | 11 | 40 | 15 | 76 | 0.53 |
| 94 | | | 1 | 1 | 17 | 2 | 21 | 0.12 |

TABLE 23—*Continued.*

| PEDIGREE NUMBER | "COMPLETE" MARGINED | "HALF" MARGINED | "SLIGHT" MARGINED | "DOTTED" MARGINED | SELF-COLORED | WHITE FLOWER | TOTAL | WHITE-MARGINED SELF-COLORED |
|---|---|---|---|---|---|---|---|---|
| 9 | 5 | | 13 | 15 | 37 | | 70 | 0.89 |
| 18 | 2 | 6 | 3 | 4 | 14 | | 29 | 1.07 |
| 44 | | 2 | 2 | 2 | 7 | | 13 | 0.86 |
| 62 | 3 | 6 | 27 | 28 | 73 | | 137 | 0.88 |
| 96 | 2 | 2 | 3 | 5 | 11 | | 23 | 1.09 |
| 7 | 3 | 9 | 7 | 21 | 33 | 14 | 87 | 1.21 |
| 17 | 6 | 5 | 2 | 8 | 25 | 21 | 67 | 0.84 |
| 38 | | 3 | 4 | 6 | 11 | 8 | 32 | 1.18 |
| 55 | 2 | 3 | 2 | 1 | 11 | 7 | 26 | 0.73 |
| 66 | 10 | 16 | 8 | 19 | 38 | 22 | 113 | 1.39 |
| 12 | 3 | 7 | 10 | 3 | 13 | | 36 | 1.77 |
| 30 | 8 | 15 | 22 | 23 | 21 | | 89 | 3.24 |
| 64 | 1 | 4 | 5 | 6 | 2 | | 18 | 8.00 |
| 90 | 1 | 3 | 13 | 3 | 9 | | 29 | 2.22 |
| 20 | 2 | 1 | 5 | 1 | 4 | 10 | 23 | 2.25 |
| 32 | 2 | 3 | 5 | 2 | 2 | 2 | 16 | 6.00 |
| 35 | 5 | 4 | 6 | 4 | 8 | 15 | 42 | 2.38 |
| 53 | 2 | 2 | 3 | 1 | 4 | 7 | 19 | 2.00 |
| 63 | 3 | 3 | 4 | 6 | 2 | 4 | 22 | 8.00 |

* Bold face figures indicate the character of the respective parental $F_2$.

97. The $F_3$ expectation of the white-margined $F_2$ flowers is (1) one breeding true, (2) four throwing one-fourth self-colored flowers, (3) four making segregation into 9 white-margined and 7 self-colored flowers, in every 16 trials. Nineteen $F_2$ used for $F_3$ treatment had all flowers with an incomplete margin. Consequently their $F_3$ was expected to be either (2) or (3), and the results actually proved the case. Though the ratio of white-margined flowers *versus* self-colored ones in every pedigree, as indicated in table 23 is, to some extent, variable, still it can be divided into two categories; one the segregating ratio of 3:1 and the other the segregating one of 9:7.

TABLE 24

*Summarized data of 9 pedigrees expected to have segregated in the 3:1 ratio.*

| | WHITE-MARGINED | SELF-COLORED | TOTAL |
|---|---|---|---|
| Total | 191 | 65 | 256 |
| *Expected* | *192* | *64* | *256* |

In a tentative attempt, 9 pedigrees of numbers 12, 20, 30, 32, 35, 53, 63, 64 and 90 may be regarded to have segregated in the former ratio. If we omit the segregants of white flowers in this calculation, the total data will be as shown in table 24.

Thus the result will perfectly accord with the expected numbers calculated on the basis of a 3:1 ratio, if only one individual would occur in the other column. Those pedigrees, however, which are considered to have segregated in a 9:7 ratio are numbers 7, 9, 17, 18, 38, 44, 55, 62, 66 and 96, ten in number, and their total data are shown in table 25 with the white-flowered segregants omitted.

TABLE 25

*Summarized data of 10 pedigrees expected to have segregated in the 9:7 ratio.*

| | WHITE-MARGINED | SELF-COLORED | TOTAL |
|---|---|---|---|
| Total | 265 | 260 | 525 |
| *Expected* | *295.31* | *229.69* | *525* |

Thus the pedigrees segregating in a 3:1 ratio and of those assorting in a 9:7 one are 9 and 10, respectively, in number. This almost completely coincides with the expectation, where an equal ratio was foresighted. In regard to the "complete"-margined flowers, two $F_2$ plants were examined with their progeny, which were allowed to grow in the same seedling bed, together with those of white flowers, but no self-colored flowers were found among them, all flowers that came out day after day having completely white-margined corollas.

Out of 78 self-colored $F_2$, 26 threw some white-margined flowers in the subsequent generation, but the remaining 52 bred true to the type. On this occasion, the expectation is 37 pure pedigrees against 18 segregating ones, or, in other words, about a 2:1 ratio is expected, and the result was actually so. According to the expectation, the items of the segregating families should be 2:8:8 or 1:4:4 of (1) segregating into self-colored and white-margined flowers in a 3:1 ratio, (2) segregating into the same phenotypes in 13:3, and (3) segregating in the same classes in 55:9 as in the case of $F_2$. In these three segregating types, the proportion of the white-margined flowers to the self-colored ones is 0.33, 0.23 and 0.16,respectively. These figures practically so resemble one another that we can hardly draw a distinct line among them by looking at the $F_3$ data. But evidence in favor of this occurrence can be obtained in another way of estimation. Thus, if 26 self-colored $F_2$, which threw some white-margined flowers, are

of these three segregating types in the ideal ratio, the white-margined flowers to the self-colored ones is estimated to be 0.212, in the total proportion, and the actual average ratio calculated on the basis of the total results of 26 pedigrees is 0.296, which is a confirmation, and not the rejection, of our hypothesis.

In the foregoing pages, the white flower segregants were often omitted in statement. But if a linkage occurs in the relation between the factor $x$ and one of the three factors concerning the white margin, we should have a more or less misleading result for accurate discussion. Such, however, was not the case, the data supporting the view of independent inheritance.

## THE OCCURRENCE OF A SPECIAL FACTOR WHICH VARIES THE QUANTITY OF THE WHITE MARGIN

### *The result of cross 324×71–2*

In a cross between 324, a pedigree having white margin on its corolla, and 71–2, a pedigree of white flowers with a colored stem, white-margined flowers were produced as $F_1$. The degree in development of the white margin was "slight" in these hybrids. And we have the following record for $F_2$:

TABLE 26

*$F_2$ data of cross 324×71–2.*

| PEDIGREE | "COMPLETE"-MARGINED | "HALF"-MARGINED | "SLIGHT"-MARGINED | "DOTTED"-MARGINED | SELF-COLORED | WHITE FLOWER | TOTAL |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 1 | 5 | 14 | 22 | 10 | 52 |
| 2 | 1 | 1 | 12 | 16 | 23 | 19 | 72 |
| 3 | 1 | 1 | 4 | 8 | 11 | 8 | 33 |
| 4 | 2 | 5 | 18 | 35 | 50 | 35 | 145 |
| 5 | 5 | 8 | 18 | 21 | 10 | 24 | 86 |
| 6 | 1 | 5 | 13 | 11 | 11 | 10 | 51 |
| Total | 10 | 21 | 70 | 105 | 127 | 106 | 439 |

Of six families, four, from 1 to 4, were cultivated in our experimental garden of the Agricultural College, TOKYO IMPERIAL UNIVERSITY, while the other two, 5 and 6, were grown on a private farm at Komaba. They all segregated about one-fourth of white flowers, and still contained a number of self-colored flowers. In regard to the contents of the colored flowers, the ratio of white-margined flowers to the self-colored ones varies considerably in different families; namely:

TABLE 27

*Varied proportions of white-margined flowers to self-colored ones in the $F_2$ families.*

| PEDIGREE | WHITE MARGINED FLOWER : SELF-COLORED FLOWER | | |
|---|---|---|---|
| 1 | 0.91:1 | | |
| 2 | 1.30:1 | | |
| 3 | 1.27:1 | 1.17:1 | |
| 4 | 1.20:1 | | 1.62:1 |
| 5 | 5.20:1 | 3.90:1 | |
| 6 | 2.73:1 | | |

The ratios of the families, 1 to 4, are relatively low, being close upon one another, while those of families, 5 and 6, however, are very high, the average being 1.17:1 and 3.90:1 respectively. From the point that these ratios correspond almost exactly to 9:7 (=1.29:1) and 3:1, respectively, some may accept the occurrence of different segregating ratios in different families which are derived from the same parents. But this is quite incorrect. It can hardly be thought that these unexpected results are due to the heterozygotic nature of the parents, because both parental pedigrees have been selfed through generations, and are considered each as a constant race, though one parent, 71–2, being a white flower, the behavior of the factors for the corolla pattern cannot be detected with its phenotype, which fact might admit of doubt. The variability of the ratio is more evident in $F_3$. The average ratio of the white-margined flowers to the self-colored ones in 18 $F_3$ pedigrees, (see table 28), which produced some self-colored flowers, is 11.51, being much larger than 1.62, the average value of $F_2$. The $F_3$ plants were raised on a private farm leased at Yoyogi, a neighboring division of Komaba, in 1923. The conspicious difference in the average values of $F_2$, therefore, is attributable to the difference of the plots on which the pedigrees were grown. This fact may throw light on the special nature of a factor now dealt with. An unexpected result also came out from an examination of the progeny of self-colored $F_2$, which, according to our experience, should either breed true or throw some white-margined flowers in the subsequent generation. Actually their offspring, without an exception, consisted of a great number of white-margined flowers. The test was made with 15 $F_2$ plants, and the results were quite clear of the unusual behavior, due to the fluctuating manifestation of a corolla pattern. Then, another question will arise as to whether all variations witnessed here in degree of the white margin are also due to a mere fluctuation or not. Though there may have existed a considerable fluctuation in this connection, the variation is attributed, to some extent, to the segregation of a definite factor as the basis of the whole variability.

TABLE 28

*$F_3$ data of cross 324×71–2.*

| PEDIGREE NUMBER | "COMPLETE"-MARGINED | "HALF"-MARGINED | "SLIGHT"-MARGINED | "DOTTED"-MARGINED | SELF-COLORED | WHITE FLOWER WITH COLORED STEM | TOTAL | AVERAGE PATTERN VALUE | WHITE-MARGINED/SELF-COLORED |
|---|---|---|---|---|---|---|---|---|---|
| 23 | 18 | 12* | 6 | | | | 36 | 3.33 | 100.00 |
| 4 | 6 | 18 | 2 | 2 | | | 28 | 3.00 | 100.00 |
| 6 | | | 1 | | | | 1 | 2.00 | 100.00 |
| 9 | 7 | 8 | 0 | | | | 15 | 3.47 | 100.00 |
| 17 | 16 | 12 | 5 | | | | 33 | 3.33 | 100.00 |
| 45 | 29 | •15 | 3 | 2 | | | 49 | 3.45 | 100.00 |
| 8 | 6 | 12 | 5 | 2 | 4 | 12 | 41 | 2.48 | 6.25 |
| 26 | 13 | 21 | 28 | 6 | 3 | 20 | 91 | 2.49 | 22.67 |
| 41 | 4 | 12 | 15 | 5 | 1 | | 37 | 2.35 | 36.00 |
| 19 | 15 | 13 | 10 | 0 | | | 38 | 3.13 | 100.00 |
| 21 | 2 | 14 | 1 | 1 | | | 18 | 2.94 | 100.00 |
| 27 | | 4 | 2 | 1 | 1 | | 8 | 2.13 | 7.00 |
| 28 | | 2 | 5 | 1 | | | 8 | 2.13 | 100.00 |
| 30 | 20 | 8 | 3 | 1 | | | 32 | 3.47 | 100.00 |
| 39 | 8 | 21 | 3 | 0 | | | 32 | 3.15 | 100.00 |
| 1 | 1 | 4 | 3 | 5 | 2 | 3 | 18 | 1.80 | 6.50 |
| 2 | 6 | 3 | 3 | 3 | | 7 | 22 | 2.80 | 100.00 |
| 12 | | | 3 | 4 | | 1 | 8 | 1.43 | 100.00 |
| 14 | 8 | 13 | 9 | 3 | 1 | 6 | 40 | 2.71 | 33.00 |
| 22 | 1 | | 4 | 1 | | 2 | 8 | 2.17 | 100.00 |
| 33 | 2 | 5 | 7 | 1 | | 3 | 18 | 2.53 | 100.00 |
| 25 | | 6 | 13 | 3 | 3 | | 25 | 1.88 | 7.33 |
| 29 | | 9 | 5 | 1 | 0 | | 15 | 2.53 | 100.00 |
| 35 | 1 | 1 | 3 | 6 | 9 | | 20 | 0.95 | 1.22 |
| 38 | 11 | 6 | 15 | 20 | 9 | | 61 | 1.83 | 5.78 |
| 46 | | 1 | 3 | | 0 | | 4 | 2.25 | 100.00 |
| 47 | | | 1 | 3 | 2 | | 6 | 0.83 | 2.00 |
| 3 | | | 1 | 1 | 1 | 1 | 4 | 1.00 | 2.00 |
| 5 | 2 | 6 | 4 | 3 | 1 | 8 | 24 | 2.31 | 15.00 |
| 11 | 3 | 11 | 15 | 8 | 5 | 14 | 56 | 1.98 | 7.40 |
| 13 | 1 | 10 | 9 | 8 | 1 | 4 | 33 | 2.07 | 28.00 |
| 18 | 1 | 1 | 4 | 3 | 1 | 5 | 15 | 1.80 | 9.00 |
| 20 | 4 | 8 | 16 | 17 | 11 | 18 | 74 | 1.59 | 4.09 |
| 31 | 4 | 8 | 16 | 19 | 4 | 20 | 71 | 1.78 | 11.75 |
| 40 | 1 | 3 | 4 | 11 | 9 | 9 | 37 | 1.14 | 2.11 |
| 44 | 4 | 6 | 16 | 4 | 0 | 11 | 41 | 2.33 | 100.00 |
| Total of 11 pedigrees | | | | | | 312 | 312 | | |

* Bold face figures indicate the character of the respective parental $F_2$.

A comparison is made between the degree of the parental $F_2$ and the average value of their progeny in table 29.

TABLE 29

*Average value of the $F_3$ pedigrees in each pattern.*

| DEGREE AND VALUE OF WHITE MARGIN OF $F_2$ | NUMBER OF $F_3$ PEDIGREE | AVERAGE VALUE |
|---|---|---|
| Absence (0) | 15 | 1.82 |
| "Dotted" (1) | 12 | 2.53 |
| "Slight" (2) | 8 | 2.82 |
| "Half" (3) | 1 | 3.33 |
| "Complete" (4) | .. | .. |

A definite correlation exists between them, and their condition tells us of the occurrence of a factor which plays some rôle in the variation of degree of the white margin. Consequently, the self-colored flowers, which appeared in $F_2$, are not the results of a mere fluctuation of the ordinary white-margined flowers, but they are due to the failure in manifestation of the corolla pattern as a result of fluctuation of the plants having the genetic constitution for a little quantity of white margin. The little quantity of the corolla pattern here is an effect of a special factor, which is novel to us. The above table indicates a tendency that the $F_2$ flowers, which have a large quantity of white margin, have generally the corresponding genetic constitution. But the fact that the white-margined flowers are much higher in their proportion to the self-colored ones in $F_3$ than to that of $F_2$, and that each $F_3$ pedigree is generally higher in the average value than its corresponding parental $F_2$, represents how strongly the environment affected on the general manifestation of the factor in question. Such conspicuous variability is also found in the pattern value of the $F_3$ families as is given in table 30.

TABLE 30

*Varied pattern values of $F_2$ families.*

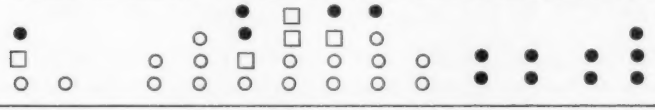
| PEDIGREE | PATTERN VALUE | | |
|---|---|---|---|
| 1 | 0.64 | | |
| 2 | 0.89 | | |
| 3 | 0.92 | 0.83 | |
| 4 | 0.85 | | 1.05 |
| 5 | 1.63 | 1.52 | |
| 6 | 1.37 | | |

Here again a condiderable difference is recognized between the pedigrees of Nos. 1 to 4 and those of Nos. 5 and 6, which were raised on different

farms as already observed in the ratio of white-margined flowers to self-colored ones. The average pattern value of the former is 0.83, while that of the latter 1.53, the difference between the two ratios being attributed to the diversity of the environments under which the plants were raised. And further, if we take the $F_3$ data the variability is more remarkable, the variation table as regards the frequency distribution of the pattern value of the $F_3$ pedigrees being indicated in table 31.

TABLE 31

*Frequency distribution of $F_2$ in regard to the quantitative variation of white margin.*



| 0.7 | 0.9 | 1.1 | 1.3 | 1.5 | 1.7 | 1.9 | 2.1 | 2.3 | 2.5 | 2.7 | 2.9 | 3.1 | 3.3 | 3.5 | 3.7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

Pattern value

In the above table, the symbol of ○ denotes $F_2$ segregated white flowers, while the one homozygous for the color producing factor is designated by ●. The $F_2$ represented by the symbol of □ gave only colored flowers, but the progeny is too small in number to ascertain whether it is homozygotic or heterozygotic in this respect. The frequency distribution represents a mountain with modal classes neighboring the mean value (2.32), and suggests the occurrence of a small mountain on each side. The result is thus no longer a single curve, but an aspect of frequency distribution resembling the variation curve developed on the basis of a 1:2:1 ratio of monohybrid. Such conspicuous variation cannot be attributed to a mere fluctuation, because the $F_3$ plants were raised on the same plot under the same treatment of cultivation. We may take this opportunity to explain the hereditary behavior of the corolla pattern by giving a factor, $F^l$, which reduces the quantity of the white margin in its development. The manifestation by this factor in the corolla pattern, however, is very variable in its nature, though, in the main, weakening in the development of the pattern when the plant contains the $F^lF^l$ factors. The white margin of plants heterozygotic for this factor may fluctuate forward and backward, in a moderate degree, in the representation of their trait. Some fluctuation has been observed in the manifestation of the corolla pattern even in the $F^aF^aF^bF^bf^lf^l$[5] plants, according to the nature of the environments of the cultivation, the time of record, etc., the pedigrees

[5] The white-margined flowers usually carry such a genetic constitution.

making up the right mountain in the variation table are probably no more than the fluctuating representation of this genotype.[6] So the environment, in which $F_2$ was reared and recorded, greatly reduced by degrees the development of the corolla pattern. The self-colored $F_2$ flowers were not only $F^aF^aF^bF^bF^lF^l$ genotype, but they contained some $F^aF^aF^bF^bF^lf^lf^l$, the $F_3$ data representing the mixture of the different genotypes in the self-colored flowers. In this connection, we may expect that the average pattern value of the pedigrees, in which appearance of the self-colored flowers is high in percentage, are lower, on the whole, than those of the low pedigree whether they are the same or not in their genetic constitution. Six $F_2$ families are the same in their factor complex, but they differ often very widely one from another. Notwithstanding their wide variability, there exists a good plus correlation between the frequency in appearance of self-colored flowers and the average pattern value. The same is true with the $F_3$ data, table 32 being given for this evidence.

TABLE 32

*Data showing a fair plus correlation between pattern value and proportion of white-margined flowers to self-colored ones.*

| AVERAGE PATTERN VALUE | NUMBER OF PEDIGREE | AVERAGE PROPORTION OF WHITE-MARGINED FLOWER TO SELF-COLORED ONE |
|---|---|---|
| Below 1 | 3 | 1.74 |
| 1–2 | 7 | 6.66 |
| 2–3 | 8 | 19.41 |

In this case, we have again a plus correlation between them. An additional statement may be written in this connection. Of the $F_3$ pedigrees, those throwing no self-colored flowers are not so confined as to $f^lf^l$ constitution, so their average pattern value may not be remarkably high, though higher, on the whole, than that of the pedigrees mixed with the self-colored flowers. In practice, we had the results noted in table 33.

TABLE 33

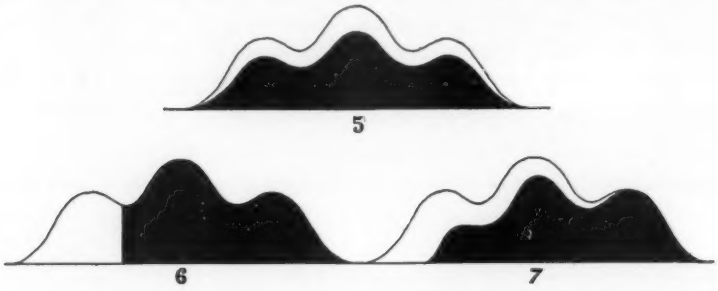
*Average pattern values of white-margined pedigrees segregating self-colored flowers and those breeding true.*

| GENETIC TYPE OF WHITE-MARGINED PEDIGREE | NUMBER OF PEDIGREE | AVERAGE PATTERN VALUE |
|---|---|---|
| Pedigree breeding true | 18 | 2.75 |
| Pedigree segregating self-colored flower | 18 | 1.90 |

The data prove this expectation. The evidence above-mentioned will serve to show the behavior of the factor $F^l$.

[6] We cannot deny the presumed occurrence of any modifier in this case.

The one fact that cannot be overlooked is a remarkable difference in the average pattern value between the $F_3$ pedigrees segregating white flowers and those giving only colored flowers. When the pedigrees containing below ten individuals were impartially omitted, the homozygotic and heterozygotic ones for the color producing factor were both 14 in number. The average pattern value of the former is 2.84, while that of the latter only 2.13, the difference seeming to be too large to be left unnoticed. Roughly speaking, this difference cannot be attributed to the effect of the environment which played an important rôle in different ways with different pedigrees, because they were raised in the same plot under uni-




FIGURES 5 to 7. The trimodal curves were diagrammatically figured by arranging the theoretical frequency of variation in the degree of the corolla pattern in the white-margined $F_2$ flowers based on a 1:2:1 ratio. The white part represents the distribution of the white flowers (in which the degree of the corolla pattern cannot be phenotypically recognized, but the figures are only theoretically attempted), while the black part is applied to that of the colored flowers. Figures 5, 6 and 7 show the cases of independent segregation, complete and partial repulsions, respectively.

form treatments and their record taken almost at the same time. This difference is recognized also by inspecting the variation table given on a former page. The frequency distribution of the homozygotic and heterozygotic colored flowers conspicuously differed one from the other. The members of the small mountain on the right-hand side supposed to have the $f^1f^1$ constitution consisted only of homozygotes for the factor $X$, while the contrary is the case with the members of the mountain on the left, though the latter is not so remarkable as the former. From such evidence, we may conclude that the factor $f^1$ links with $c$ (IMAI 1921), the factor for the white flower with a red stem. The $F_2$ plants, which were recognized to carry the $f^1f^1$ constitution, were all homozygous in regard to the factor $C$, and consequently the linkage in this case is a repulsion scheme, which is also expected by the parental constitution. The source of the factor $F^1$

must be searched after 71–2, because its partner 324 had a complete margin on its corolla.

When the factors *f'* and *c* make an independent segregation, the distributive frequency of the white flowers in $F_2$ should be as represented in figure 5, where the members are equally distributed over a three-modal curve, and accordingly the variation curve of white margin in the colored flowers is not affected in its quality. When a complete repulsion takes place between them, the $F_1$ should give only two sorts of gametes, *Cf'* and *cF'*, so the result of $F_2$ is $1CCf'f'+2CcF'f'+1ccF'F'$. This formula, if shown graphically, will be as given in figure 6, where the distribution of the colored flowers is limited to two mountains, but the other is occupied by the

TABLE 34

*Data for classification of results by which linkage calculation may be attempted.*

| | CC | | | | | | Cc | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F'F'* | | *F'f'* | | *f'f'* | | *F'F'* | | *F'f'* | | *f'f'* | |
| | Pedigree number | Pattern value | Pedigree number | Pattern value | Pedigree number | Pattern value | Pedigree number | Pattern value | Pedigree number | Pattern value | Pedigree number | Pattern value |
| Frequency | 35 | 0.95 | 6* | 2.00 | 4 | 3.00 | 3* | 1.00 | 1 | 1.80 | | |
| | 48* | 0.85 | 25 | 1.88 | 9 | 3.47 | 12† | 1.43 | 2 | 2.80 | | |
| | | | 27* | 2.13 | 17 | 3.33 | 40* | 1.14 | 5 | 2.31 | | |
| | | | 28* | 2.13 | 19 | 3.13 | | | 8 | 2.48 | | |
| | | | 29 | 2.53 | 21 | 2.94 | | | 11 | 1.98 | | |
| | | | 38 | 1.83 | 23 | 3.33 | | | 13 | 2.07 | | |
| | | | 41 | 2.35 | 30 | 3.47 | | | 14 | 2.71 | | |
| | | | 46* | 2.25 | 39 | 3.15 | | | 18 | 1.80 | | |
| | | | | | 45 | 3.45 | | | 20 | 1.59 | | |
| | | | | | | | | | 22* | 2.17 | | |
| | | | | | | | | | 26 | 2.49 | | |
| | | | | | | | | | 31 | 1.78 | | |
| | | | | | | | | | 33 | 2.53 | | |
| | | | | | | | | | 44 | 2.33 | | |
| Total pedigrees containing many individuals | 1 | | 4 | | 9 | | 1 | | 13 | | 0 | |
| Total pedigrees containing many individuals | 1 | | 4 | | 0 | | 2 | | 1 | | 0 | |
| Grand total | .. | | .. | | .. | | 3 | | 14 | | 0 | |

* Those containing a few individuals.

† This pedigree entered on the left side of middle mountain in the variation table, but it may be more properly put into this class by its segregating aspect.

members of the white flower. As a result, a quite asymmetrical curve in regard to the distribution of the colored flowers is obtained. While the linkage is moderately high, the frequency of the colored flowers may be somewhat asymmetrical, but it is represented by a trimodal curve or the like, as figure 7, due to the unbalanced distribution of the white flowers. To which of these does the result of the present cross correspond? As figured in table 31, the observed frequency of the corolla pattern in the white-margined $F_2$ flowers is represented by a somewhat trimodal curve but, strictly speaking, the left mountain is meagre, and resembles the figure 7. With a view to determining the value of linkage quantitatively, table 34 is arranged by inspecting the result of frequency of the white-margined $F_2$ flowers.

The classification cannot be as precise as wished for, owing to a conspicuous fluctuation in the manifestation of the factor *F'*, but as it is based on the average pattern value of each pedigree and the segregating condition of the degree of the corolla pattern, the table will show an outline in this connection. Of 36 pedigrees, 19 segregated white flowers, while the rest did not. It may be a little premature to accept that all the latter are homozygous as to the color producing factor, because it contains some pedigrees which consisted of a few individuals. When these pedigrees (containing less than ten individuals) are omitted in calculation, we have 14 in number. In comparison of numbers between the homozygotic and heterozygotic pedigrees, such meagre ones must be omitted from the counting, also in the latter, for the sake of impartiality in this connection. Thus we have 14 pedigrees of both homozygotic and heterozygotic colored flowers. These numbers are deviated from the expected ratio of 1:2, but they may be regarded as a chance result. Owing to this considerable deviation, an examination in the contents of genotypes may be separately attempted. Of the 14 pedigrees homozygotic to the color producing factor, only one has the *F'F'* constitution, 4 the *F'f'* and the 9 remaining are considered to have the *f'f'*. In his case, the segregating scheme being a repulsion, four sorts of gamete of *CF*, *Cf*, *cF* and *cf* produced by $F_1$ should be formed in a ratio of 1:x:x:1 when the nature of unequal gametic production is represented by x (x>1). The pedigrees homozygotic to the factor *C*, therefore, are expected theoretically to be: *F'F'* 1, *F'f'* 2x and *f'f'* $x^2$. If the observed numbers cited above are applied to those figures, we shall have such values as x=2 from the relation of *F'F'* and *F'f'*, x=3 from *F'F'* and *f'f'*, and x=4.5 from *F'f'* and *f'f'*, or the average of x=3.17. If the same attempt is made with the pedigrees heterozygotic to the color producing factor, the result will be as

follows: In this examination, however, our consideration is not prevented from being based on 17 pedigrees of the total quoted in the table, which contains some meagre pedigrees. Of these, $F^lF^l$ is 3, $F^lf^l$ 14 and $f^lf^l$ 0, while, theoretically, the first is expected to be 2x, the second $2x^2+2$ and the last 2x. In these data, we have $x=4.66$ from the relation of the former two. If we venture to make an average between this value and the former, 3.17, the result is $x=3.92$ or the value of x is practically 4. This indicates that the gametic ratio between the factors *c* and $f^l$ is 1:4 or, in other words, 20 percent of the crossovers. This linkage value does not necessarily indicate an accurate intensity of the real occurrence, because the estimation was not based on fully reliable data. So the value has to be accepted as a rough estimation of the linkage between *c* and $f^l$.

*The result of cross 50×71–2*

One of the parents used in this case being different from that of the former cross, while the other is similar in both cases, the data of the hybrid progeny gave another instance of a high fluctuation of the corolla pattern. Thus, in the present cross, 50 was used instead of 324, but the degree of the white margin in both races is the same; namely, a "complete" state. The $F_1$ plants came out to bear the "slight"-margined flowers, and the $F_2$ is composed of the following segregants:

TABLE 35

*$F_2$ data of cross 50×71–2.*

| PEDIGREE | "COMPLETE"-MARGINED | "HALF"-MARGINED | "SLIGHT"-MARGINED | "DOTTED"-MARGINED | SELF-COLORED | WHITE FLOWER | TOTAL | PATTERN VALUE | WHITE-MARGINED/SELF-COLORED |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 12 | 18 | 2 | 0 | 16 | 50 | 2.33 | 100.00 |
| 2 | 2 | 18 | 19 | 12 | 1 | 9 | 61 | 2.15 | 51.00 |
| 3 | 6 | 26 | 34 | 14 | 2 | 30 | 112 | 2.24 | 40.00 |
| 4 | 5 | 10 | 36 | 3 | 0 | 17 | 71 | 2.31 | 100.00 |
| 5 | 2 | 2 | 17 | 4 | 1 | 9 | 35 | 2.00 | 25.00 |
| 6 | 6 | 6 | 29 | 13 | 0 | 23 | 77 | 2.09 | 100.00 |
| 7 | 10 | 10 | 22 | 14 | 0 | 24 | 80 | 2.29 | 100.00 |
| 8 | 5 | 5 | 48 | 28 | 4 | 27 | 131 | 1.93 | 25.00 |
| 9 | 6 | 6 | 26 | 19 | 0 | 29 | 92 | 2.10 | 100.00 |
| Total | 44 | 115 | 249 | 109 | 8 | 184 | 709 | 2.15 | 64.63 |

These $F_2$ plants were all reared on the ground of Yoyogi in 1922. Roughly speaking, the data of the nine families are the same. The segregation of one-fourth white flowers in every family was nothing but expecta-

tion, while the meagre appearance of the self-colored flowers is worthy of note. We used 71–2 as one of the parents, so the appearance of self-colored flowers may be naturally expected in this cross, owing to the countenance of the factor $F^{l}$. The frequency of the mixture, however, was very low, and the numerical ratio of the white-margined flowers to the self-colored ones is 64.63:1 in the total number. This is hardly comparable with 1.62:1, the corresponding $F_2$ ratio in the previous cross. The special nature of the factor $F^{l}$ may account for such a result, but an additional assumption may be made with the occurrence of a modifier, which affects the function of the factor $F^{l}$. If the low frequency of the self-colored flowers is a hereditary tendency, the latter may be the case, while, if it is only a potential occurrence, the former concept is true.

The pattern value shows that the nine families are almost equally represented by fluctuating about 2.15. This is a little over twice as much as 1.05 of the corresponding value of 324×71–2. Thus the higher pattern value accompanies a higher ratio of white-margined flowers against self-colored ones. The actual frequency distribution of the $F_2$ plants in classes of the pattern degree occurred in a single curve (see the total number of table 35).

### *The results of crosses RA×71–2 and RB×71–2*

In the previous crosses, in which 71–2 were used as one of the parents, the complemental factors for the corolla pattern were homozygous in regard to their constitution. On the contrary, each one (RA or RB) of the parents of the present crosses, taken as a partner with 71–2, had self-colored flowers and consequently they have to be regarded as $f^{a}f^{a}F^{b}F^{b}f^{l}f^{l}$. In this state, $F_1$ had a very meagre quantity of white margin, and in $F_2$ they brought about the result found in table 36.

TABLE 36

*$F_2$ data of crosses RA×71–2 and RB×71–2.*

| CROSS | WHITE-MARGINED | SELF-COLORED | WHITE FLOWER WITH COLORED STEM | TOTAL |
|---|---|---|---|---|
| RA×71–2 | 71 | 81 | 62 | 214 |
| RB×71–2 | 40 | 51 | 25 | 116 |
| Total | 111 | 132 | 87 | 330 |

The record is not taken of the variation in quantity of the corolla pattern in the present crosses, and the table will not contain any classification in this respect.

In this connection, the genotypes expected in $F_2$ should be as indicated in table 37.

TABLE 37

*Theoretical genotypes and their ratio in $F_2$.*

| REFERENCE | GENOTYPE | RATIO |
|---|---|---|
| A | $F^aF^aF^bF^bf^lf^l$ | 1 |
| | $F^af^aF^bF^bf^lf^l$ | 2 |
| B | $F^aF^aF^bF^bF^lf^l$ | 2 |
| C | $F^aF^aF^bF^bF^lF^l$ | 1 |
| D | $F^af^aF^bF^bF^lf^l$ | 4 |
| E | $F^af^aF^bF^bF^lF^l$ | 2 |
| F | $f^af^aF^bF^bf^lf^l$ | 1 |
| | $f^af^aF^bF^bF^lf^l$ | 2 |
| | $f^af^aF^bF^bF^lF^l$ | 1 |

Of these classes, genotype A is invariably white-margined flowers, B is the same in the majority of the cases, though it sometimes assumes self-colored flowers, C and D are expected to mix together in a greater number of self-colored flowers than in the former case, and lastly E is most conspicuous in the production of self-colored flowers. The plants grouped in class F are certainly all self-colored flowers whatever their outer conditions may be. The proportion of the total numbers of this class is 25 percent, but the practical percentage may be enlarged by absorbing the number of the self colored flowers produced by the fluctuation of the classes B, C, D and E. A, however, is the only class that contains plants invariably blooming with a white-margined corolla, and their proportion to the total number is 18.75 percent. Practically the percentage of the mixture of the self-colored flowers should vary between 25, as its absolute minimum quantity, and 81.25 its maximum quantity, according to the nature of the environment, and the actual proportion was 54.32 percent which indicates nearly an intermediate value between the two limits. Of 54.32 percent, 25 percent is due to the results in segregation of the factor $f^a$, or of the plants carrying a constitution for blooming invariably self-colored flowers (corresponding to class F). Reducing this 25 percent, from the total, we obtain 29.32 percent, which is attributed theoretically to the result in occurrence of factor $F^l$. These $F_2$ were raised in the neighboring plot of $F_2$ pedigrees, 5 and 6, of 324×71–2 on the ground of Yoyogi under

the same treatment. Roughly speaking, the two may be tentatively interpreted as due to the same chance fluctuation in regard to the development of the corolla pattern. The mixing proportion of the self-colored flowers in $F_2$ pedigrees, 5 and 6, of the cross 324×71–2 is 20.39 percent. For the reason that this figure represents the percentage of the failing manifestation of $F^aF^aF^bF^bF^fF^f$ and $F^aF^aF^bF^bF^ff^f$ (practically the latter concerns little in this case), the value may possibly be applied to the present cross. This 20.39 percent indicating the proportion of the false self-colored flowers among the $F^aF^aF^bF^b$ constitution, the value changed into 25 percent × 25.39 percent = 15.29 percent in these present crosses, because in them the invariable mixture of 25 percent of the self-colored flowers was expected in the segregation of the factor $f^a$. The figure 15.29 percent represents the failing proportion in the manifestation of the white-margined flower to the total in the effect of the factor $F^f$. Practically, however, the subject is made more complicated by the segregation of the factor $f^a$. B and C are the only classes concerning this factor alone. The proportion of the plants having such a constitution corresponds to merely one-fourth of those in the cross 324×71–2. So the figure 15.25 percent must be divided by four, the result being 3.82 percent, which should be regarded as the failing proportion in the effect of the factor $F^f$ alone. Thus the value concerning the classes B and C is tentatively determined. If we add this figure to 25 percent, the percentage of the properly self-colored flowers, we have 28.82 percent, the reduction of which from the total proportion of 54.32 percent leaves 25.50 percent, the corresponding proportionn of the co-working effect of the factors $f^a$ and $F^b$. The items of the self-colored flowers in regard to their origin may be summed up in table 38.

TABLE 38

*Classification of self-colored $F_2$ flowers in respect to the probable cause of their appearance.*

| PROBABLE CAUSE BY WHICH SELF-COLORED $F_2$ PLANTS WERE PRODUCED | RELATIVE PERCENTAGE | ABSOLUTE PERCENTAGE |
|---|---|---|
| Self-colored proper | 25.00 | 46.02 |
| Produced by the effect of $F^f$ alone | 3.82 | 7.03 |
| Produced by the co-working effect of $f^a$ and $F^f$ | 25.50 | 46.95 |
| Total | 54.32 | 100.00 |

Between the cases in which only the factor $F^f$ is affected and in which both factors $f^a$ and $F^f$ are concerned, we have a conspicuous difference in the percentage of their quantity. This indicates a greater chance of production in the self-colored flowers heterozygotic to the factor $f^a$ compared

with the homozygotic $F^aF^a$. But we cannot directly compare the failing proportion of these two cases with their figures, because their mixing ratio is different in $F_2$. Theoretically the homozygotic and heterozygotic plants for the factor $F^a$ are found in a 1:2 ratio among $F_2$. In comparison, we preliminarily divide 25.50 percent (the failing proportion of the latter) by two, and if the quotient 12.75 percent is compared with 3.83 percent (the failing proportion of the former), it proportionates in 3.38:1. This figure indicates an absolute difference in the failing proportion in the manifestation of the white-margined flower between the two classes above cited.

I wish to acknowledge my indebtedness to Professor K. MIYAKE, under whose direction this investigation was made, to Mr. K. HASHIMOTO for his warm encouragement so kindly given, and to Messrs. B. KANNA and K. TABUCHI for their help in the present experiment. The expenses needed in the study were partly defrayed by a grant from the IMPERIAL ACADEMY, to which my cordial thanks are expressed on this opportunity.

## SUMMARY

1. The white margin behaves, in most cases, as a simple dominant to the self-colored condition in inheritance. In $F_2$ of such crosses, segregation took place at a 3:1 ratio, and the results of the next generation were quite in accordance with expectation. The hybrid flowers have white margins to the degree of "half" or nearly so.

2. In most cases, the segregating ratio was recorded as 3:1, while in some crosses it occurred just in accordance with 1:2:1. In the latter cases, a sharp discontinuous segregation of homozygotic and heterozygotic white-margined flowers seems to occur in their manifestation.

3. The segregation of the self-colored flowers from the white-margined ones in the ordinary cases concerns the $F^a$, $f^a$ allelomorphs.

4. The manifestation of the white margin, however, is not simple in some cases. The complete production of the white pattern is effected by two factors of $F^a$ and $F^b$. Neither factor can produce by itself the marking on the corolla, acting complementally.

5. The fact that the white margin links very closely with *contracta* is already pointed out by IMAI (1919, 1925) and HAGIWARA (1922a, 1926). According to the estimation of linkage value the frequency of crossing over is about 1 percent. In this linkage, the factors concerned are $f^a$ and $d$ (*contracta*).

6. The other complemental factor $F^b$ links with $n_n$, the factor for

*Nandina*. The frequency of crossing over between them is about 0.5 percent, or actually 0.39 percent.

7. In the combination of $F^a$ and $f^b$ the flower generally assumes full coloration, but when they enter into *Nandina* leaves their manifestation is often a "dotted"-margined corolla. So, the "dotted"-margined flowers of *Nandina* may be usually different from those of non-*Nandina* in their factor constitution.

8. The quantity of the white margin varies considerably according to the genetic constitution, whether homozygotic or heterozygotic for the pattern factor or factors. In a normal culture the manifestation of various factor combinations is estimated in table 13.

9. In a cross we met with the segregation of three factors, which concerned the production of the white margin; namely, two complemental factors and their inhibitor. The third factor $F^h$, as studied by TAKEZAKI (1916) and HAGIWARA (1922b, 1926), entirely suppresses the manifestation of the complemental factors, resulting in a self-colored flower. Under this circumstance, the ratio of white-margined and self-colored flowers in $F_2$ is 55:9. The results of $F_2$ and $F_3$ were quite in accordance with expectations developed by our hypothesis.

10. Our experience tells the fact that the self-colored flowers, which, produced in the hybrid progeny, should either breed true or throw some white-margined flowers in the subsequent generation, while we had quite exceptional cases, in which offspring, without an exception, consisted of a great number of white-margined flowers. This peculiar nature of inheritance is due to the fluctuating manifestation of a partial inhibitor $F^l$.

11. The factor $F^l$ suppresses partially the formation of white margin, but the effect is very variable according to the environment under which the plants were raised. As the effect of this factor, the segregating ratio of white-margined and self-colored flowers varies in a considerable extent by the production of false self-colored flowers.

12. The partial inhibitor links moderately (about 20 percent) with *c*, the factor for the white flower with colored stem (IMAI 1921).

13. When the factor $f^a$ is segregated simultaneously with the factor $f^l$ the proportion of the self-colored flowers in $F_2$ should vary between 25 percent, as its absolute minimum quantity, and 81.25 percent, its maximum quantity, according to the nature of environment. In a cross, we actually obtained 54.32 percent of this proportion.

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# ON THE VARIABILITY OF A WHITE-EARED FORM IN *AMARANTUS PANICULATUS*

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TABLE OF CONTENTS

| | PAGE |
|---|---|
| INTRODUCTION | 242 |
| Results of experiments | 242 |
| Genetic types of "striped form" | 244 |
| Behavior of red-eared individuals | 244 |
| On the appearance of mosaic-eared individuals | 248 |
| SUMMARY | 252 |
| LITERATURE CITED | 252 |

## INTRODUCTION

*Amarantus paniculatus* is an ornamental plant belonging to Amarantaceae, but it is comparatively less common in our gardens. The ordinary form of this plant bears dark red leaves and produces rich magenta inflorescence in autumn. A rare form, which may be called "white-eared," and with which the present investigation has been carried on, has reddish leaves, but it blooms in white inflorescence. Though the ears are thus said to be white, they are magenta in color when they are young and afterwards fade out to be almost white. We obtained a white-eared form in about 1917, but the press of work prevented us from investigating it in a systematic breeding and some years had elapsed without much crop. Its peculiar behavior in transmission, however, attracted our notice and we started an experiment with its pedigree culture, in 1922, when a white-eared specimen made luxuriant growth in our experimental plot. This individual was bicolored in white and "red" (magenta), and produced eleven ears, seven of which were white, while the rest were red. Starting with this specimen, we expected at first to determine the hereditary behavior of the vegetative variation. Our experiment was thus started with a single vigorous plant, because we had learned, from a preliminary breeding, that the white-eared form habitually produced mosaic ears and that quite red-eared individuals appeared frequently in its progeny.

## RESULTS OF EXPERIMENTS

From a mosaic-eared specimen, we raised offspring separately for each

ear and succeeded in obtaining the result as is shown in table 1. The white ears gave progeny mostly containing whites, and did not always breed

TABLE 1

*The result of experiment in 1923.*

| PARENTAL BRANCH | ITS CHARACTER | RED-EARED | MOSAIC-EARED | WHITE-EARED | TOTAL | PERCENT RED-EARED | PERCENT MOSAIC-EARED |
|---|---|---|---|---|---|---|---|
| I | Red-eared | 8 | 2 | 39 | 49 | 16.33 | 4.08 |
| II | " | 2 | 4 | 107 | 113 | 1.77 | 3.54 |
| VIII | Nearly red-eared | 14 | 15 | 135 | 164 | 8.54 | 9.15 |
| XI | " | 5 | 9 | 42 | 56 | 8.94 | 16.07 |
| Total | | 29 | 30 | 323 | 382 | 7.59 | 7.85 |
| III | White-eared | 2 | 2 | 17 | 21 | 9.52 | 9.52 |
| IV | Nearly white-eared | 0 | 0 | 24 | 24 | 0.00 | 0.00 |
| V | " | 0 | 2 | 44 | 46 | 0.00 | 4.35 |
| VI | " | 1 | 0 | 26 | 27 | 3.70 | 0.00 |
| VII | White-eared | 1 | 4 | 46 | 51 | 1.96 | 7.84 |
| IX | " | 4 | 9 | 113 | 126 | 3.17 | 7.14 |
| X | Nearly white-eared | 12 | 5 | 73 | 90 | 13.33 | 5.56 |
| Total | | 20 | 22 | 343 | 385 | 5.19 | 5.71 |
| Grand total | | 49 | 52 | 666 | 767 | 6.39 | 6.78 |

true to the type. They had a mixture of red-eared and mosaic-eared individuals in the average proportion of 5.19 percent and 5.71 percent, respectively. Not only was such an unusual result the case with the white-eared progeny, but red ears also produced red-eared and mosaic-eared plants in the average proportion of 7.59 percent and 7.85 percent, respectively. The breeding results of white and red ears of the original plant were the same, for the most part, from which fact we can see that the varied red part on the white-eared form does not undergo a genetic change. The mosaic ear is bicolored generally in red and white, though sometimes with an intermediate color. In this paper, however, we shall not give the intermediate color in question, as it was treated as "white" in our record.

The third generation was raised in 1924, and table 2 contains a summary of the results then obtained. A close inspection of the table shows the fact: Whatever the ear color of the parental plant or branch may be, the progeny contains some red-eared and mosaic-eared individuals. Special attention is drawn to the progeny of red-eared plants, of which a simple segregation of 3 red-eared and 1 white-eared or mosaic-eared may be

expected in their offspring. Quite contrary to our expectation, however, the red-eared specimens gave only a common result. They proved to be no mutants, but genetically white-eared.

### GENETIC TYPES OF "STRIPED FORM"

Many "striped forms" of different species offered a genetic subject. Among them, we may cite the striped flowers of the snapdragon studied by DE VRIES (1905) and of Mirabilis investigated by CORRENS (1910), the variegated maize reported by EMERSON (1914) and the mosaic comb of the cockscomb observed by TERASAWA (1922). These "striped forms" are not the same in their genetic behavior, and so, IMAI (1924) gathered them into two types as follows:

Type I—The self red variegation on the plant body is due to the vegetative mutation of a colorless factor to a red one. So the progeny of the self red part consists of red and striped forms in a simple ratio. (Examples: DE VRIES' snapdragon, EMERSON'S maize.)

Type II—The occurrence of the self red part is due to the so-called pseudo-mutation, and not to a real factor change, the variegation being confined merely to the changing manifestation of the character. Such differentiation in character, however, is entirely reduced into the original form in passing gametogenesis or its mother tissue, and so the vegetative variation in this case does not affect the contents of its offspring. (Examples: CORRENS' Mirabilia, TERASAWA'S cockscomb.)

Which of these two types will be the case with our mosaic Amarantus? As was stated in the foregoing section, the red ears on the white form is not accompanied by any factorial change and we may confirm this with type II. This is quite correct when we consider only the offspring of the mosaic individuals, but a further consideration of the genotype of red individuals, which appeared in the progeny of white and red ears, will show the reason why the confirmation cannot be made so simply. The red individuals of the "striped form" in both types I and II owe their appearance to a factorial mutation, but this is not the case with our Amarantus, which, therefore, falls under neither type I nor II, but its presence demands another type or type III.

### BEHAVIOR OF RED-EARED INDIVIDUALS

The red individuals of the plant belonging to type I or II are regarded to be mutants, and so they produce progeny containing about three-

fourths of red specimens. On fifteen red individuals of our Amarantus, we raised their offspring containing about 14 percent of red specimens, as is shown in table 2. The proportion of the red specimens was somewhat high, but majority of their offspring were white-eared as is generally the case with this plant. The red Amarantus in question is quite clear in existence to the eye, but its presence does not stand on a proper genotype!

TABLE 2

*The result of experiment in 1924.*

| IN 1922 | IN 1923 | | PEDIGREE | RED-EARED | MOSAIC-EARED | WHITE-EARED | TOTAL | PERCENT RED-EARED | PERCENT MOSAIC-EARED |
|---|---|---|---|---|---|---|---|---|---|
| Red-eared branch | Red-eared individual | | I-R1 | 5 | 3 | 15 | 23 | 21.74 | 13.04 |
| | | | XI-2 pedigrees | 2 | 0 | 19 | 21 | 9.52 | 0.00 |
| | | | VIII-R1 | 16 | 8 | 43 | 67 | 23.88 | 11.94 |
| | | | II-2 pedigrees | 19 | 7 | 72 | 98 | 19.39 | 7.14 |
| | | | Total | 42 | 18 | 149 | 209 | 20.10 | 8.61 |
| | Mosaic-eared individual | Red part | I-M2 | 0 | 0 | 5 | 5 | 0.00 | 0.00 |
| | | | XI-M1 | 7 | 5 | 68 | 80 | 8.75 | 6.25 |
| | | | VIII- 3 pedigrees | 35 | 12 | 145 | 192 | 18.23 | 6.25 |
| | | | II- 4 pedigrees | 12 | 10 | 113 | 135 | 8.89 | 7.71 |
| | | | Total | 54 | 27 | 331 | 412 | 13.11 | 6.55 |
| | | Mosaic part | XI-M1 | 2 | 1 | 42 | 45 | 4.44 | 2.22 |
| | | | VIII-7 pedigrees | 105 | 52 | 396 | 553 | 19.00 | 9.40 |
| | | | Total | 107 | 53 | 438 | 598 | 17.89 | 7.17 |
| | | White part | I-2 pedigrees | 0 | 0 | 7 | 7 | 0.00 | 0.00 |
| | | | XI-8 pedigrees | 19 | 14 | 568 | 601 | 3 19 | 2.31 |
| | | | VIII-3 pedigrees | 13 | 18 | 145 | 176 | 7.39 | 10.23 |
| | | | II-4 pedigrees | 17 | 23 | 405 | 445 | 3.82 | 5.17 |
| | | | Total | 49 | 55 | 1125 | 1229 | 3.99 | 4.49 |
| | | Totals | | 210 | 135 | 1894 | 2239 | 9.38 | 6.03 |
| | White-eared individual | | I-18 pedigrees | 13 | 12 | 288 | 313 | 4.15 | 3.83 |
| | | | XI-23 pedigrees | 7 | 14 | 411 | 432 | 1.62 | 3.24 |
| | | | VIII-22 pedigrees | 60 | 62 | 829 | 951 | 6.31 | 6.52 |
| | | | II-26 pedigrees | 16 | 38 | 1020 | 1074 | 1.49 | 3.54 |
| | | | Total | 96 | 126 | 2548 | 2770 | 3.50 | 4.55 |
| | Grand total | | | 348 | 279 | 4591 | 5218 | 6.67 | 5.35 |

TABLE 2 (continued)

| IN 1922 | IN 1923 | | PEDIGREE | RED-EARED | MOSAIC-EARED | WHITE-EARED | TOTAL | PERCENT RED-EARED | PERCENT MOSAIC-EARED |
|---|---|---|---|---|---|---|---|---|---|
| White-eared branch | Red-eared individual | | X- 5 pedigrees | 4 | 0 | 74 | 78 | 5.13 | 0.00 |
| | | | IX- 4 pedigrees | 11 | 3 | 98 | 112 | 9.82 | 2.68 |
| | | | Total | 15 | 3 | 172 | 190 | 7.09 | 1.58 |
| | Mosaic-eared individual | Red part | III-M1 | 119 | 1 | 61 | 180 | 65.75 | 0.55 |
| | | | X- 2 pedigrees | 46 | 2 | 43 | 91 | 50.55 | 2.20 |
| | | | IX- 4 pedigrees | 17 | 13 | 85 | 115 | 14.78 | 11.30 |
| | | | Total | 182 | 16 | 189 | 387 | 47.03 | 4.13 |
| | | Mosaic part | III- 2 pedigrees | 57 | 46 | 109 | 212 | 26.89 | 21.70 |
| | | | VII- 4 pedigrees | 7 | 134 | 367 | 508 | 1.83 | 26.38 |
| | | | V-M2 | 9 | 4 | 36 | 49 | 18.37 | 8.16 |
| | | | X-M3 | 1 | 0 | 40 | 41 | 2.44 | 0.00 |
| | | | IX- 4 pedigrees | 13 | 7 | 128 | 148 | 8.79 | 4.73 |
| | | | Total | 87 | 191 | 680 | 958 | 9.08 | 19.93 |
| | | White part | III-M1 | 14 | 0 | 48 | 61 | 22.95 | 0.00 |
| | | | VII-M1 | 5 | 52 | 231 | 188 | 1.74 | 18.06 |
| | | | V-M1 | 8 | 1 | 50 | 59 | 13.56 | 1.70 |
| | | | X- 4 pedigrees | 20 | 12 | 248 | 280 | 7.14 | 4.29 |
| | | | IX- 7 pedigrees | 19 | 24 | 271 | 314 | 6.05 | 7.64 |
| | | | Total | 66 | 89 | 847 | 1002 | 6.59 | 8.88 |
| | Totals | | | 335 | 296 | 1716 | 2347 | 14.27 | 12.61 |
| | White-eared individual | | III- 5 pedigrees | 26 | 20 | 225 | 271 | 9.59 | 7.75 |
| | | | VII- 4 pedigrees | 21 | 285 | 558 | 864 | 2.43 | 32.99 |
| | | | IV- 8 pedigrees | 14 | 95 | 1047 | 1156 | 1.21 | 8.22 |
| | | | V- 4 pedigrees | 5 | 37 | 207 | 249 | 2.01 | 14.86 |
| | | | X-41 pedigrees | 61 | 30 | 969 | 1060 | 5.75 | 2.83 |
| | | | IX-29 pedigrees | 40 | 66 | 1289 | 1395 | 2.87 | 4.73 |
| | | | Total | 167 | 533 | 4295 | 4995 | 3.34 | 10.67 |
| | Grand total | | | 517 | 832 | 6183 | 7532 | 6.86 | 11.05 |
| | Grand totals | | | 865 | 1111 | 10774 | 12750 | 6.78 | 8.71 |

The red individuals of this plant, therefore, is said to be quite the same as the red ears of white individuals in a rough estimation; namely, both reds are due to the differentiated manifestation of the character.

TABLE 3

*The frequency distribution of deviation in the mixing proportion of red-eared individuals in pedigrees (Mean=5.53 percent).*

| Dev./P.E. | −8 | −7 | −6 | −5 | −4 | −3 | −2 | −1 | 0 | +1 | +2 | +3 | +4 | +5 | +6 | +7 | +8 | +9 | +10 | +11 | +12 | +17 | +18 | +25 | +26 | +27 | +28 | +61 | +62 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Observed | 1 | 2 | | 5 | 7 | 39 | 61 | 31 | 26 | 12 | 8 | 4 | 4 | 4 | 2 | 3 | | 1 | 2 | 1 | | 2 | | 1 | | 1 | | 1 | |
| | 3 | | | | | | | | | | | | | 18 | | | | | | | | | | | | | | | |
| *Expected* | *0.109* | | *0.654* | *3.924* | *14.606* | *35.207* | *54.500* | *54.500* | *35.207* | *14.606* | *3.924* | *0.654* | | *0.109* | | | | | | | | | | | | | | | |

$\chi^2 = 3163.058$ P=0.000

TABLE 5

*The frequency distribution of deviation in the mixing proportion of mosaic-eared individuals in pedigrees. (Mean=5.67 percent).*

| Dev./P.E. | −7 | −6 | −5 | −4 | −3 | −2 | −1 | 0 | +1 | +2 | +3 | +4 | +5 | +6 | +7 | +8 | +9 | +10 | +11 | +12 | +13 | +14 | +15 | +16 | +17 | +18 | +23 | +24 | +51 | +52 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Observed | 1 | | | | 7 | 32 | 62 | 32 | 21 | 25 | 11 | 9 | 5 | 2 | | | 2 | | 1 | | 2 | | 1 | | 1 | 1 | | 1 | | 1 |
| | 1 | | | | | | | | | | | | | 12 | | | | | | | | | | | | | | | | |
| *Expected* | 0.109 | | *0.654* | *3.924* | *14.606* | *35.207* | *54.500* | *54.500* | *35.207* | *14.606* | *3.924* | *0.654* | | 0.109 | | | | | | | | | | | | | | | | |

$\chi^2 = 1409.854$ P=0.000

With the result of 1923, we calculate the frequency in production of red individuals to be 6.39 percent, and with that of 1924 we have 6.78 percent, the average mixing proportion being 6.75 percent. The total individuals recorded in these two years are 13517 in number, with which the average proportion was calculated, so the above figure can be regarded as a mean value of the mixing proportion of red individuals. The proportion of the reds, however, varies somewhat considerably in different progenies. We raised the offspring of 237 pedigrees, of which 19 pedigrees containing meagre progeny, we preliminarily omitted them from the present estimation. If we calculate the quotient of deviation by probable error in each segregating pedigree and make a variation table with their frequencies the result will be table 3. The deviation of frequencies is very remarkable and their distribution is not symmetrical, the frequencies being rather crowded on the minus side, while they are scattered up to very high classes on the plus side. As a result, the value of P is so low that we cannot regard the variation to be a chance deviation. Such a marked variation in frequency is not only the case, in which the unit was taken by a pedigree, but nearly the same variation can be seen in the data, in which the progeny of each ear is considered separately for one plant. Then, to what cause is this marked variation due? It is clear that this does not stand on a sort of genetic factor, which manifests a distinct character and is transmitted with certainty. Our hypothesis, therefore, which we hope will explain the matter, is tentatively offered on this opportunity.

The red individuals,[1] which appeared in the progeny of white, mosaic and red plants, have the origin of their self-redness in their parental gametogenesis. The frequency of proportion throwing red individuals is governed by "potency." In general, our Amarantus carries weak "potency." Its constancy is not strong, but it is frequently transformed into strong "potency" in somatogenesis. A varied cell propagates on the plant tissues after its successive divisions and may produce a mass of cells carrying strong "potency." If the varied cell is located in the growing point of the stem, there may be produced a large region of strong "potency" on the plant body, or pure ears for this "potency" occur.

Thus we explain the variable frequency for the production of red individuals with two conditions of strong and weak "potencies." If the region of strong "potency" is large, the frequency will be high, while, if this is small, the proportion will fall down. In such conditions, the variation in mixing proportion of reds in pedigrees will be far deviated. In high-

[1] We do not mean that such is the case with all, but at least with most of them. Why we write such a note may be understood from the discussion made in the next section.

proportioned pedigrees, the red individuals will be produced *en masse*, and give some distortion to the variation curve. If we inspect table 2 closely, we shall find a marked difference in the average frequency of offspring between red-eared individuals, and red and mosaic ears of mosaic-eared ones on one side, and white-eared plants and white ears of mosaic-eared ones on the other; the former is 17.68 percent, while the latter only 3.78 percent. The low frequency of the latter may be due to the condition that the transformation from weak to strong is partly stopped in the white tissues. The white ears are thus handicapped in their frequency, but there are observed some high pedigrees for the production of reds, as can be seen in table 4. The parental white ears of these pedigrees seem to carry somewhat a large region of strong "potency."

TABLE 4

*High proportionated pedigrees in regard to the appearance of red-eared individuals.*

| PEDIGREE | ITS PARENTAL CHARACTER | RED-EARED | MOSAIC-EARED | WHITE-EARED | TOTAL | PERCENT RED-EARED |
|---|---|---|---|---|---|---|
| II-M1 | White ear of mosaic specimen | 9 | 0 | 8 | 17 | 52.94 |
| VIII-W18 | White-eared specimen | 24 | 3 | 32 | 59 | 40.68 |
| IX-M2 | White ear of mosaic specimen | 12 | 6 | 15 | 33 | 36.36 |
| X-W36 | White-eared specimen | 8 | 1 | 9 | 18 | 44.44 |

The strong "potency," however, which propagated in sporogenesis, is reduced to its prototypic weak condition in gametogenesis or in its mother tissue, but it manifests its effect by giving red gametes at a high proportion. So the embryos make their start with weak "potency," but its transformation into strong occurs habitually in the different stages of their development. This transformation seems to be so frequent that most of the plants may experience it during their plant growth.

## ON THE APPEARANCE OF MOSAIC-EARED INDIVIDUALS

The red individuals, which appeared in the progeny of the white form, owe their origin to the gametic pseudo-mutation, and the mosaic ones are produced by somatic occurrence. So these two types differ in one generation in regard to their period, in which they originated, the former being one generation ahead of the latter. If the somatic variation occurs in the embryonic development or seedling stage of the white-eared individuals, and further, if the varied cell is located just in the growing point of the plumule or the young stem, red-eared individuals may be produced. Such may be the actual case, but, at the same time, it seems that we cannot consider all or many of the reds to have developed with

such a vegetative origin. For, in some pedigrees, notwithstanding the fact that the red specimens were very abundantly produced, we have only a few mosaic ones. In view of these facts we may regard most red-eared plants as having a gametic origin.

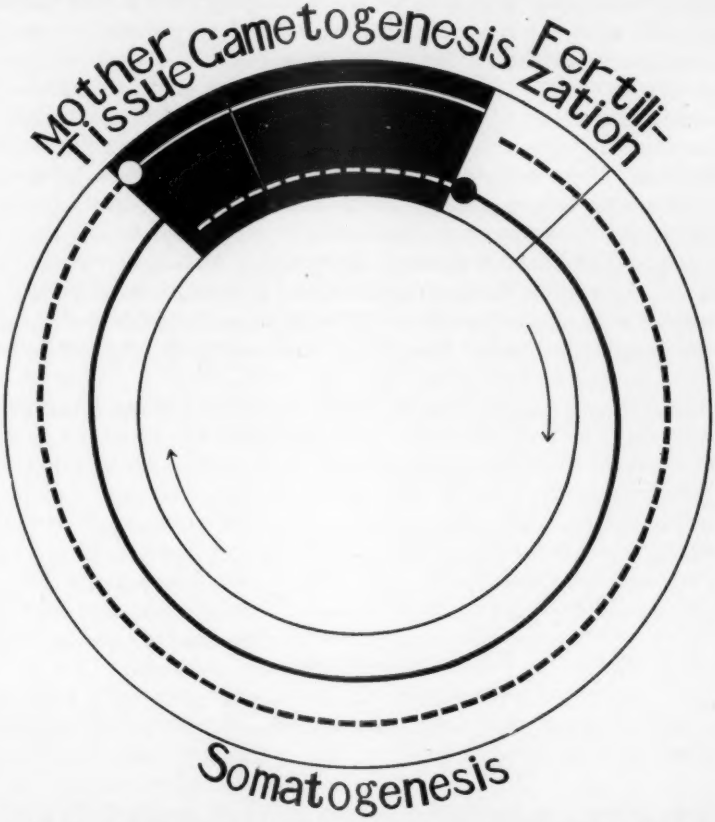


FIGURE 1.—Diagram denoting the behavior of two types of "potency." The outer line shows type A, and the inner one represents type B. Their latent period is drawn in dotted line, while active period in solid one.

The frequency in production of mosaic-eared individuals is 6.78 percent in 1923 and 8.71 percent in 1924, the proportion being thus roughly fixed. Their average proportion, therefore, is 8.60 percent, which is very near 6.75 percent, the average mixing proportion of red individuals. For the

reason that the mosaic individual is nothing but a modified form of a white one, we can calculate the proportion of individuals making vegetative variation from the number of mosaics and of the total mosaics and whites. Such proportions are 7.24 percent in table 1 and 9.35 percent in table 2, the average being 9.23 percent. The proportion is thus roughly fixed, but when the consideration is made for each pedigree, we find a conspicuous variability in their frequencies, as in the case of red plants (see tables 1 and 2). Table 5 is showing variation of the frequencies of mosaic pedigrees, which were reared in 1924, in regard to the departure from the mean value. The nature of variation is quite similar to that of red individual. Some might call attention to the much-discussed "potency" to account for this variability. If the case is simply due to the parallel effect of the same "potency," we should expect sufficient correlation in frequency between red and mosaic individuals. As is shown in the correlation table (table 6), the frequencies are scattered here and there in the space formed by a diagonal, which is drawn as a long side of a triangle to the proportional lines. The correlation is low ($r = 0.121 \pm 0.045$), but

TABLE 6

*Correlation table of the mixing proportion between red and mosaic individuals in pedigrees.*

| Percent of mosaics | 0 | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 | 21 | 23 | 25 | 27 | 29 | 31 | 33 | 35 | 37 | 39 | 41 | 43 | 45 | 47 | 49 | 51 | 53 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 41 | | 1 | 1 | | | | 1 | | | | | | | | | | | | | | | | | | | | | |
| 39 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 37 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 35 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 33 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 31 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 29 | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 27 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 25 | | 1 | | 1 | 1 | | | | | | | r=0.121±0.045 | | | | | | | | | | | | | | | | |
| 23 | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 19 | | 1 | | | | 1 | | | | | 1 | | | | | | | | | | | | | | | | | |
| 17 | | 3 | | 1 | 1 | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | |
| 15 | | 2 | | | | | 1 | | | | | | | | | | | | | | | | | | | | | |
| 13 | | 1 | | 1 | 1 | | | | | | | 1 | | | | | | | | | | | | | | | | |
| 11 | | 2 | | | 1 | | | 1 | 1 | 1 | | | 2 | | | | 1 | | | | | | | | | | | |
| 9 | | 9 | 1 | 3 | 5 | 2 | | 1 | | | | 1 | | 1 | | | | | | | | | | | | | | |
| 7 | | 4 | 1 | 3 | 1 | 1 | | 1 | 2 | 1 | 1 | 1 | 1 | 1 | | | | | 1 | | | | | | | | | |
| 5 | | 12 | 2 | 2 | 5 | | 1 | 1 | 1 | | | | | | | | | 1 | | | | 1 | | 1 | | | | |
| 3 | | 11 | 1 | 7 | 1 | | | 1 | | | | | | | | | | | | | | | | | | | | |
| 1 | | 5 | 1 | 1 | | 2 | | | 1 | | | | | | | | | | | | | | | | | | 1 | |
| 0 | | 34 | 7 | 9 | 8 | 6 | 8 | 2 | | 3 | 1 | 2 | | | | | | | | | | | | | | | | 1 |

*Percent of reds*

it indicates some possibility of the parallel effect of the "potency." When, however, we study the relation of both proportions for each pedigree we shall find not infrequently the pedigrees, in which red and mosaic specimens were very unbalancingly produced. So the case is actually more complicated. In our opinion, if we dare say, the transformation of weak "potency" to the strong condition is not confined with the tissues of plant body, but another chance is given in the mother tissue of the gametogenesis. In the latter case, the changed "potency" may be propagated and distributed *en masse* in germ cells, and as a result, mosaic specimens will appear in different proportions, containing some cases of considerable high ratios, in different pedigrees. Thus the changed "potency," which developed in the mother tissue of gametogenesis, governs the frequency of the appearance of the mosaic plants ("potency" of type B), while that developed in the tissues of the general somatic growth handles the frequency of the production of red gametes ("potency" of type A). Two types of "potency" may seemingly differ from each other in their product, the one giving the mosaic plants, and the other red ones. But the mosaics are due each to a red cell in its origin, and the reds are started each from a red gamete, so the real product is a red cell in both cases. The main difference between two types, therefore, lies in unparallelism of the latent and active periods of "potency" throughout the life cycle of the plant, the respective periods being quite mutually replaced. The latent period just mentioned denotes the time, during which the transformation of "potency" may occur in its quality and the changed cell should propagate without giving any apparent effect, while the settlement of its effect is made in the active period. The accompanying diagram will assist us in understanding the behavior of the two types of "potency."

The weak correlation above-mentioned indicates the relation of the two types of "potency," the activity of type A seeming partly to stimulate the variability of type B. And, as was stated in the foregoing section, the transformation of "potency" of type A is partly stopped in the white tissues, while it seems not to be the case with type B.

We wish to express our hearty thanks to Professor K. MIYAKE, under whose direction these experiments were carried out, and to Mr. K. HASHIMOTO for the warm encouragement so kindly given. The expenses needed in the investigation were defrayed partly by a grant from the IMPERIAL ACADEMY.

SUMMARY

1. The white-eared form of *Amarantus paniculatus* gives mosaic-eared and red-eared individuals at the average proportion of 8.60 percent and 6.75 percent, respectively, in its offspring.

2. Nearly the same result is obtainable in the progeny of red ears of mosaic individuals. So the vegetative variation is not accompanied by any factorial change.

3. From the fact that the vegetative variation owes its change to the differentiated manifestation of a character, we may confirm our Amarantus with the cases of the striped flowers of *Mirabilis* and of the mosaic comb of the cockscomb, from a genetic point of view. Actually, however, the red individuals of this plant also give nearly the same result, or, in other words, they carry no red factor. So the genetic type of our Amarantus is quite peculiar.

4. The mixing proportion of red individuals in each pedigree varies so much that we cannot attribute the variability to a mere deviation. The "potency" hypothesis tentatively suggested by us may explain the unusual results.

5. The red-eared individuals seem to have a gametic origin at least in most cases.

6. The frequency of the production of mosaic individuals varies considerably, as in the case of red-eared individuals, when we take pedigrees, one by one, into consideration. But we cannot attribute this variability simply to the parallel effect of the same "potency" cited above, because the result reveals some complication. We suggested, therefore, two chances for transformation in the quality of "potency."

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# THE GENETICS OF A VIVIPAROUS TOP-MINNOW PLATYPOECILUS; THE INHERITANCE OF TWO KINDS OF MELANOPHORES[1]*

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TABLE OF CONTENTS

| | PAGE |
|---|---|
| INTRODUCTION | 253 |
| Taxonomy of Platypoecilus | 254 |
| Native habitat and variability of the wild fishes | 256 |
| Varieties and their color patterns | 259 |
| The Stippled variety | 261 |
| The Spotted variety | 262 |
| The Red variety | 263 |
| The Black variety | 263 |
| The Gold variety | 265 |
| Cultural methods | 265 |
| Experimental data | 267 |
| The stocks used | 268 |
| The cross of Gold by Red | 268 |
| Direct: Gold ♀ by Red ♂ | 268 |
| Reciprocal: Red ♀ by Gold ♂ | 269 |
| The cross of Gold by Stipple | 275 |
| GENERAL DISCUSSION | 275 |
| SUMMARY | 277 |
| LITERATURE CITED | 278 |

## INTRODUCTION

Although there have been only a few truly genetical studies of fishes, they have already contributed at least two new concepts to the science

[1] Paper No. 150, Department of Plant Breeding, CORNELL UNIVERSITY, Ithaca, New York.

The work was conducted under the joint supervision of Professor A. C. FRASER and Professor G. C. EMBODY. To them I wish to express my thanks for laboratory equipment, information from frequent discussions and constant encouragement. Professor H. D. REED has led the research which has resulted in finding the morphological equivalents of the genetic factors. My thanks are also due to Dr. R. G. HARRIS, director of the Biological Laboratory, for placing at my disposal such equipment as was necessary for me to carry the experiments through the summer of 1925 at Cold Spring Harbor, Long Island.

* The GALTON AND MENDEL MEMORIAL FUND contributes the accompanying colored plate prepared from a painting by Miss LILA DVORAK.

of heredity. The first instance of crossing over in the X and Y chromosomes was demonstrated in fishes, almost simultaneously by SCHMIDT, 1920, by WINGE, 1922a, 22b, 23, and by AIDA, 1921. Secondly, the two types of sex-linkage have been reported as operating within the same sub-family; the XY type in Lebistes (SCHMIDT, 1920; WINGE, 1922a, 22b, 23) and the ZW in Platypoecilus (BELLAMY, 1922)—both genera within the sub-family Poeciliinae, the viviparous top-minnows. AIDA, 1921, has also reported the XY type of sex-linkage in Aplocheilus, a killifish belonging to the same order as the above genera. This paper deals with *Platypoecilus maculatus* and supports the hypothesis that the ZW type of sex-linkage operates in this species.

## TAXONOMY OF PLATYPOECILUS

Under the genus Platypoecilus, proposed by GÜNTHER in 1866, there have been listed, at various times, the following species:

| | |
|---|---|
| *P. couchia* | (Girard 1859) |
| *P. maculatus* | Günther 1866 |
| *P. mentalis* | Gill 1876 |
| *P. quitzeoensis* | Bean 1898 |
| *P. variatus* | Meek 1904 |
| *P. nelsoni* | Meek 1904 |
| *P. perugiae* | Everman and Clark 1906 |
| *P. dominicensis* | Everman and Clark 1906 |
| *P. tropicus* | Meek 1907 |

The first to lose its position under the genus, Platypoecilus, was *P. quitzeoensis* when MEEK (1904) saw its identity with the genus Zoogoneticus and called it *Z. quitzeoensis*.

In 1907 REGAN further reduced the list of species by combining *P. variatus* with *P. maculatus*. In addition he showed that *P. mentalis* and *P. nelsoni* were synonyms of *Poecilia sphenops* Cuv. & Val.; later, 1913, he found it necessary to refer *Poecilia sphenops* to *Mollienisia*.

REGAN (1913) brought into general taxonomic employment what HUBBS (1924) termed "by far the most valuable of all the characters which have been used in the classification of these little fishes, namely, the structure of the gonopodium (this is the intromittent organ of the male, formed by the elongation and singular elaboration of rays 3, 4, and 5 of the anal fin)." On this basis REGAN was able to refer *P. perugiae* to *Limia vittata* (Gouchenot) and *P. dominicensis* to *Limia*. At the same time *P. tropicus* was found identical with *Mollienisia sphenops*.

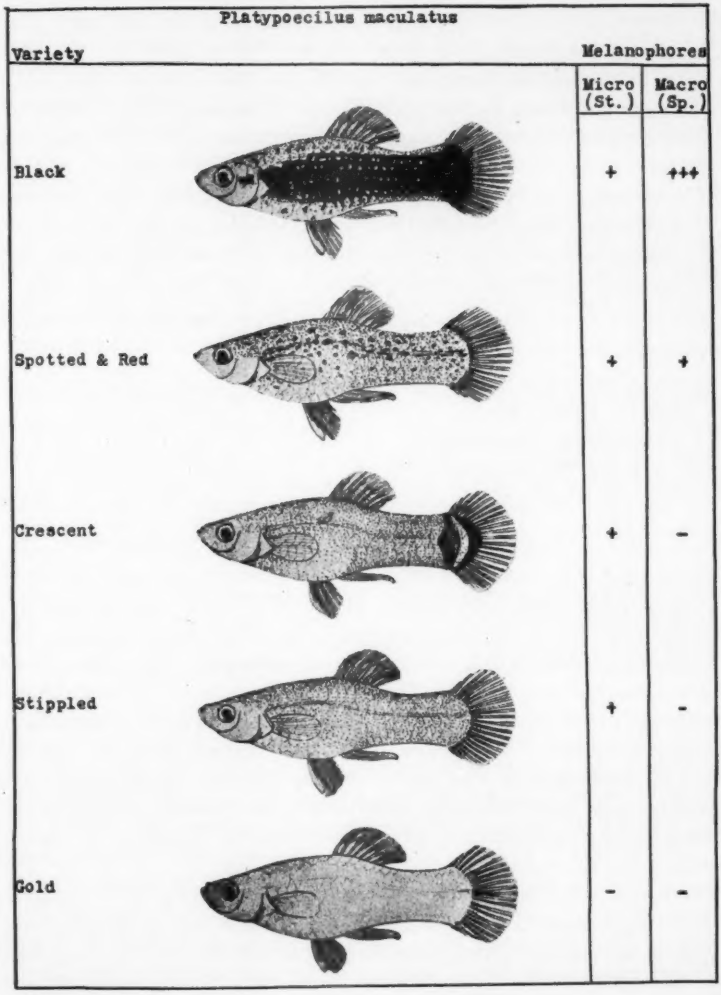



FIGURE 1.—A few varieties of *Platypoecilus maculatus*, (males) 1.4 natural size. The Red, Stippled and Gold were used in these studies. The pen and ink drawings were made by Miss MARY MEKEEL.

Two species now remain: *Platypoecilus couchia* and *P. maculatus*. The synonymy of the former is as follows:

*Limia couchia* Girard, Proc. Acad. Phila., 1859, p. 116
*Poecilia couchii*, Günther, Cat. vi, 1866, p. 347
*Poecilia couchiana*, Jordan and Gilbert, Synopsis, 1882, p. 348
*Platypoecilus couchianus*, Regan, Proc Zool. Soc. Lon. 1913, p. 977
*Platypoecilus couchia*, Gordon, Genetics **12**: p. 256

The synonomy of *Platypoecilus maculatus* is as follows:

*Platypoecilus maculatus* Günther, Cat. vi, 1866, p. 350
*Poecilus maculatus*, Regan, Bio. Cen. Amer. Pisces, 1907, p. 105
*Platypoecilus maculatus*, Regan, Proc. Zoo. Soc. Lon. 1913, p. 977

According to the latest revision (HUBBS 1924) the two species will be listed under the following groups:

Order: Cyprinodontes
Family: Poeciliidae
Sub-family: Poeciliinae
Tribe: Xiphophorini
Genus: Platypoecilus.

## NATIVE HABITAT AND VARIABILITY OF THE WILD FISHES

Platypoecilus is confined to Mexico, being most often collected in the southern, tropical fish-faunal zone. The most extensive field studies were made by MEEK (1904), who has made the following statement: "For example of extreme individual variation of color markings, I would refer to *Platypoecilus maculatus*." This also has been the concerted opinion of all those who have collected it. Although guarding against unnecessary splitting of varieties into species, MEEK nevertheless described one new one; this latter species was placed into synonomy. It is of particular importance, in these studies in inheritance and variation, to know that this top-minnow has been singled out from the total number of 227 species described by MEEK from Mexico for its variability.

The first live specimens were imported into Germany in 1907 (GERLACH 1909[1]). The aquarists there, in describing the newly arrived fishes practically redescribed the types MEEK mentioned and illustrated. They have, however, added to the list of variable types; but it must be remembered that their descriptions were made from newly imported stocks. By 1912 practically all the patterns now known had been recorded.[2] The only

[1] Determination of species mentioned, confirmed by a specialist (LEONHARDT 1909).

[2] STANSCH (1911b) and SCHREITMULLER (1911) give good summaries of varieties newly imported up to that date. FINCK (1915) lists six imported varieties.

variety which owes its identity to the influence of domestication is the Gold, described in 1920, which has, in all probability, mutated from aquarium material.

The cause of variability of Platypoecilus cannot be traced to the effect of domestication but must be sought in its native habitat. Unfortunately up to this time no systematic field studies of this species have been made, but the general situation is presented by MEEK. Under the heading, "General Topography and Hydrography of Mexico" he says:

"The greater portion of Mexico is a plateau ranging in elevation from 3000 to 8000 feet above the sea.... The northern portion is the lower and the slope is to the northeast.... The eastern border of this plateau is formed by the eastern range of the Sierra Madre mountains leaving a plain to the east of varying width.... The western Sierra is exceedingly rough and its western slope very steep."

Under "Recent Geological Changes in Mexico and their General Effect on the Fish Fauna" he says:

"Geologically speaking, within more recent times the climatic and hydrographic conditions of Mexico have been less stable than in the Mississippi Valley. The central portion of Mexico has been subject to considerable volcanic disturbances which have continued to within recent times. The northern portion has, evidently at some former time, been much better watered than it is now." Many of "the rivers of this region never reach the sea and were, perhaps formerly, the tributaries of the Rio Grande, as is indicated by the character of the fish fauna and general topography." It is also held that "the western streams have cut their way back, captured the head-waters of the eastern stream, and with them their portion of the eastern fish fauna."

The rainfall is sufficiently variable to cause seasons of wet and dry weather. In the north the rains begin about the first of May and stop about October or November, while in southern Mexico, immediately to the north of the Isthmus of Tehuantepec, from which region Platypoecilus is most often reported, the rains continue into December and occasionally extend into February.

"In the height of the wet season many of the streams overflow, forming in depressions—shallow lakes, bayous and ponds. These later become dry, causing the destruction of many fishes."

MEEK further states that the viviparous killifishes give birth to young at the end of the dry season. Since they are well developed from the start, the young are able to withstand the keen competition in the thickly populated bodies of water; when the wet season sets in, they are in better condition to become widely distributed.

Commenting upon the ceaseless environmental changes in geologic

and present periods, MEEK says: "Species of fishes in Mexico are undergoing changes, and the progress of making species of varieties is still actively in progress."

MCFARLANE (1923), after a study of the evolution and distribution of fishes believes that there is conclusive proof that Northern South America

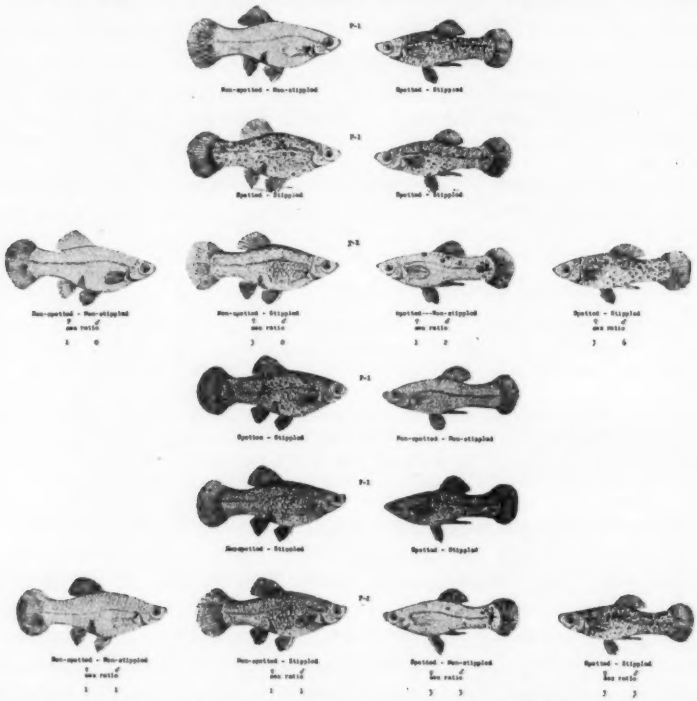


FIGURE 2.—The crosses diagrammed: above, Gold ♀ × Red ♂; below, Red ♀ × Gold ♂.

was the centre for the evolution of the Cyprinodontes, which at present are widely distributed.

Genetical studies of Platypoecilus should prove valuable in determining the nature of the variations. Individuals of a variable species may have become geographically isolated and thus may have developed several races. It will be interesting to study, thoroughly, the distribution of these fishes in their native habitat to determine the relation, if any, of a variety to its peculiar environment.

## VARIETIES AND THEIR COLOR PATTERNS

In the aquarium, where the water is maintained at subtropical temperatures, *Platypoecilus maculatus* has adapted itself as well as if it had been carried to an isolated pool in its native habitat. This interesting little fish has taken the fancy of aquarists in Germany and in the United States who have fixed a wide variety of types. Domestication has not induced the production of any new forms, with the possible exception of the Gold. Through selection it has merely brought about a splitting up of a complexly marked animal into its various components. Fifteen types, most of which have already given indication of possessing individual genetic factors, have been identified. It is here proposed that these types be named. Since data is lacking which would supply the information necessary to establish these several varieties as sub-species, it would seem best to follow the accepted method in genetical nomenclature by using common names. Corresponding with the descriptions and their respective numbers contained in the list of GORDON (1926b) the following names are offered:

| *No.* | *Name proposed* | *Synonomy* | *Pattern first described by* |
|---|---|---|---|
| 1. | Shoulder blotch | | Günther 1866 |
| 2. | Peduncular blotch | | Günther 1866 |
| 3. | Ocellus | | Meek 1904 |
| 4. | Crescent | | Meek 1904 |
| 5. | Twin blotch | | Meek 1904 |
| 6. | Dorsal black | | Gunther 1866, Meek 1904 |
| 7. | Anal black | | Gunther 1866, Meek 1904 |
| 8. | Ventrals black | | Meek 1904 |
| 9. | Barred 2, 3, 4, 5. | | Meek 1904 |
| 10. | Stippled platy | White[1] | Meek 1904 |
| 11. | Spotted platy | Pulchra[2] | Schreitmuller 1910 a, b, c. |
| 12. | Blue platy | | Schreitmuller 1910 a, b, c. |
| 13. | Red platy | Rubra[3] | Finck 1911, Stansch 1911 |
| 14. | Black platy | Nigra[4] | Holmann 1912, Gerlach 1912 |
| 15. | Gold platy[5] | Immaculatus[6] | Struve 1920, Myers 1922 |

[1] Proposed by Bellamy 1922, 1924 (see page 276 for discussion).
[2] Proposed by Boulenger, adopted by Schreitmuller 1910c.
[3] Proposed by Stansch 1911a, b.
[4] Proposed by Gerlach 1912.
[5] Proposed by Struve 1920.
[6] Proposed by Myers 1922.

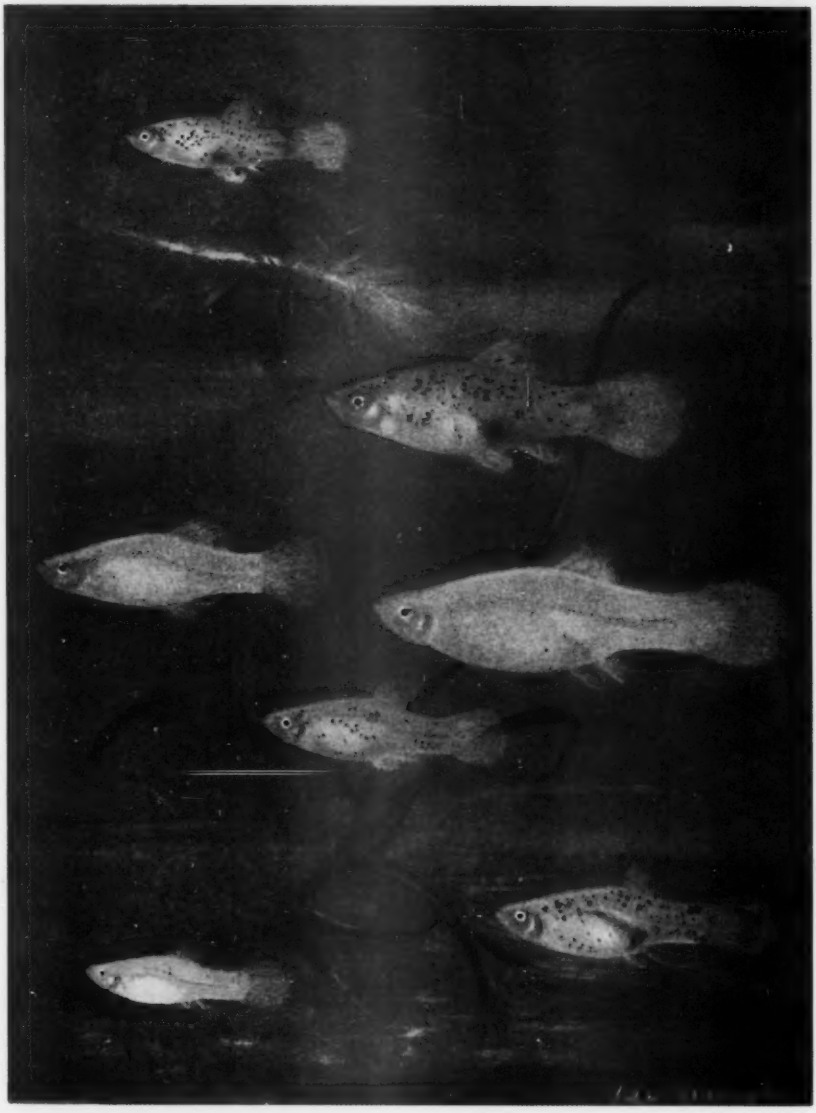
In general the varieties owe their individuality to the presence or absence of the various color pigment cells: the black or melanophore; the yellow, or xanthophores; and the red, or erythrophores. Of melano-

LEGEND FOR PLATE 1

PLATYPOECILUS MACULATUS

1. Red ♂
2. Red ♀
3. Gold ♂
4. Gold ♀
5. $F_1$ hybrid ♂: $P_1$ = Red ♂, (1) Gold ♀, (4)
   and
   $F_1$ hybrid ♂: $P_1$ = Gold ♂, (3) Red ♀ (2)
6. $F_1$ hybrid ♀: $P_1$ = Red ♂, (1) Gold ♀, (4)
7. $F_1$ hybrid ♀: $P_1$ = Gold ♂, (3) Red ♀, (2)

Figures numbered from top to bottom. Nos. 1, 5 and 6 natural size; nos. 2, 3 and 4 slightly more, No. 7 less, than natural size. No. 5 should have more red since the animal drawn was a young male.



photes there are several types,[1] a genetical study of two of which is to be presented in this report. The first and most obvious type is a large intensely black kind which occurs irregularly over the body of the animal. These cells are found in the Spotted and also in practically all of the Red varieties. When they occur in great numbers even to the point of blanketing the sides of the animal they form the Black variety. Since this type of cell is the largest, and may be seen with the unaided eye, the term macro-melanophore may be applied to it.

The next type is one which will be referred to as micro-melanophore, or commonly to be spoken of as "stipple"—stipple because of the effect of many small melanophores operating together to give a general grayish-olivacious coloration. The individuality of the pigment cells, lost to the unaided eye, may readily be observed with the aid of low magnification.

When compared under high magnification, micro- and macro-melanophores appear to differ not only in size but also in their morphology (see figures 3, 4, 5). And this is significant because long before morphological differences were discovered, genetical studies made it evident that two types of melanophores were operating: the stipple type acting as an autosomal character and spot showing sex linkage.

Recently in a preliminary study of the morphology of the melanophores a third type has been observed, a companion type to stipple. They are typically like *Ameba radiosa* when expanded (see plate 2), and appear to be due to a factor operating in a manner similar to stipple. So far every Stippled animal studied showed the third type as well as stipples.

The following varieties used in genetical work may be characterized by the following table:

| | *Red* | *Spotted* | *Black* | *Stipple* | *Gold* |
|---|---|---|---|---|---|
| Melanophores | | | | | |
| a. Stipples | + | + | + | + | − |
| b. Spots | + | + | +++ | − | − |
| Erythrophores | + | − | − | − | − |
| Xanthophores | + | + | + | + | + |

### *The Stippled variety*

The general ground color of both sexes of this type is the Dark Olive Buff of Ridgway's color chart, 1912, plate 40. This color is in the main the one most commonly seen both in this group and in other fishes. It is a highly protective type of coloration. GUNTHER (1866), for its original description, called it brownish olive; to MEEK (1904), it was olivacious; to BRIND (1919), cold straw; to BADE (1923), bluish gray to olive. This general olivaceous ground color is undoubtedly due to the presence

[1] See abstract on melanophores of Platypoecilus, GORDON (1926a).

of the dot-like micro-melanophores which occur in great numbers all over the body overlying the xanthophores. Only when the Stippled variety is observed under the low power of a binocular do the individual melanophores become clearly evident. On the mid-region immediately above the belly, these small pigment spots arrange themselves in a net-like formation, seen best in the adult females (see figure 3). Along the back, the snout, and the greater part of the tail region, the micro-melanophores are generally distributed. They are found, also, at the border of the anal fin and along all the fin rays.

In general the male is slightly brighter than the female. His dorsal fin at its origin is the Flaming Scarlet of Ridgway, plate 1. The female is generally duller and her dorsal fin is the La France Pink of Ridgway, plate 1. On the latter in the lower mid-region of the body between the anal and pectoral fins there is a brownish-black stain-like spot known to the fish fanciers as the sign of pregnancy. It is due to the black pigmented peritoneum appearing through the thin body wall at that particular place. It should rather be known as the sign of maturation, since it not only may be seen in virgin females, but in some well formed adult males as well. Other than this mark the Stippled variety when pure lacks all the patterns mentioned above. However, practically any or all patterns may be found in combination with it. As such, it becomes known under the various names of the markings.

### *The Spotted variety*

This variety was first described by SCHREITMULLER (1910b, 1910c) from newly imported aquarium specimens. Since he worked with live material his description is more complete than others previously presented, as far as coloration is concerned. Clearly being different from the typical *Platypoecilus maculatus* SCHREITMULLER (1910b) called this variety *Platypoecilus sp.*?; later BOULENGER of the BRITISH MUSEUM proposed the varietal name of *P. maculatus* var. *pulchra* and this was accepted in his 1910c paper.

The ground color of this variety is generally, as in the former, the Dark Olive Buff of Ridgway. It has a strong tendency to take on a brownish cast and often intergrades with Red in color. On the other hand it may lose some of its olivaceousness and become paler and sometimes yellowish. But the character of most significance in this variety is the presence of large black pigment spots distributed, with varying intensification, irregularly over the body. They are usually in greatest preponderance on the upper half of the body and the whole tail region, but rarely extend

to the tip of the snout, the operculum, the belly or fins. These macro-melanophores are different from the micro-melanophores, found in Stippled, although the latter pigment cells are also contained in the present variety and may be seen in parts of the body not covered by the former. The Spotted variety as such is now rarely seen in aquarium specimens, being undesirable from the breeder's point of view. All of its characteristics are preserved, however, in the Red variety which is merely the Spotted variety plus erythrophores. A fuller description of Red is given below.

*The Red variety*[1]

In this variety sexual dimorphism is well exhibited in the adult animals, but when immature, both sexes are almost indistinguishable. The adult females are generally dull, much like Spotted but often become Cinnamon Rufous of Ridgway plate 14. The males of the best strains when fully mature are the brilliant Brazil Red of Ridgway plate 1. In most cases the red begins to appear when the transformation of the anal fin into intromittent organ is almost completed. In some fishes, however, the full red color is delayed in its appearance until several months later, and many become deeply red with age. The red pigment is usually associated with a spotted type of animal, that is, most Reds in addition to possessing erythrophores, also have micro- and macro-melanophores. There is a tendency for a reduction of the melanophore number but individual variations are great and often increased numbers above the average may be found. It seems to be a general rule, however, that when macro-melanophores and erythrophores occur together, and the micro-melanophores are wanting, that there are decidedly fewer macro-melanophores present than when both kinds of melanophores are present together with erythrophores.

*The Black variety*

This variety may be considered as an extreme phase of the black spotted form. So closely set are the macro-melanophores on the sides of this variety that the spotted effect is entirely lost. A black blanket-like band of melanophores covers the animal from immediately behind the eye to the base of the caudal fin. The back, tail, belly and head regions are mostly free of macro-melanophores, but here also, as in the varieties already discussed, micro-melanophores are found. At the extremities of the black band, detached macro-melanophores are visible, showing their relation to the spotted form. The ground color of the pure Black is similar

[1] OSSWALD (1913) says that red specimens were obtained directly from Mexico.

LEGEND FOR PLATE 2

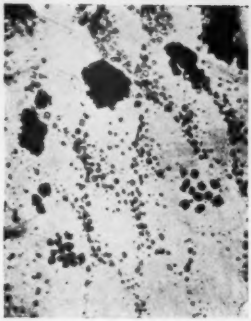
FIGURE 3.—A portion of the skin of *Platypoecilus maculatus*, under low magnification, showing spots, irregularly distributed, and stipples arranged to form a net work.

FIGURE 4.—Stipples (micro-melanophores) under high magnification: a. expanded (0.8 mm); b. partially contracted, (when fully contracted 0.3 mm.).

FIGURE 5.—Spots (macro-melanophores) under high magnification; a. expanded (2.4 mm.); b. contracted (1.2 mm.).

FIGURE 6.—The third type of melanophores adhering to the scale covering: a. expanded (0.8 mm.); b. contracted (0.2 mm.); c. entire scale under low magnification showing third type. (Note lines of growth in scales in a, b, c.).

Photo-micrographs made with assistance of Doctor D. J. LEFFINGWELL.



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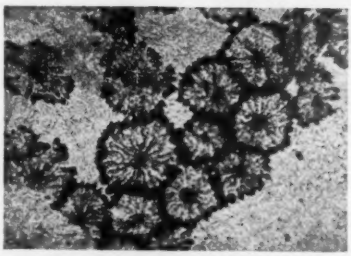

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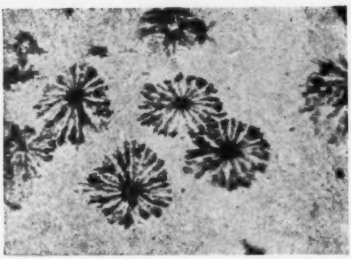
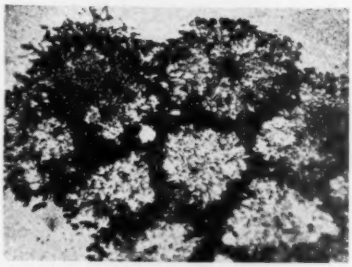


a b



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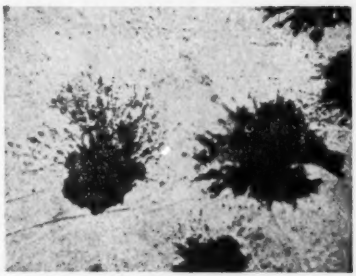
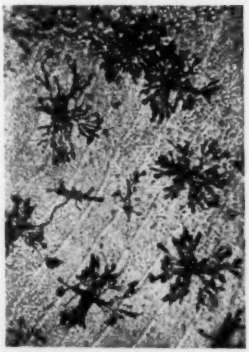
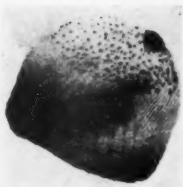


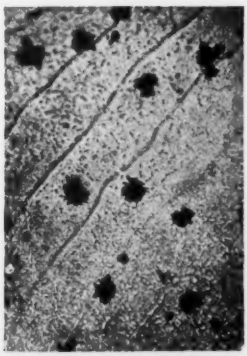
a b



6

c





b a

to that in Stippled and Spotted, generally olivaceous. When crossed with certain varieties it may become red pigmented and the scales may become iridescent as in the Blue variety (MYERS 1922).

*The Gold variety*

This variety was first described and brought to the attention of Berlin aquarists by STRUVE (1920). Sachs pointed out that the variety lacked melanophores but retained its xanthophores (1922). The latter reviewed Professor TORNIER'S experiments on Axolotl eggs to show how pigmentless forms may arise, but apparently did not believe that the same factors were operating here. MYERS (1922) also noticed the lack of melanophores in the Gold variety and remarks, "No Goldplaty the author has examined has been spotted." (Platy is often used in common terminology for Platypoecilus.) Not only is the Gold variety non-spotted but it is also non-stippled. MYERS' color description is as follows: clear transparent yellow, slightly tinged with pale orange; eyes greenish blue, dorsal fin flushed red." For standard comparison the body color may be referred to as Deep Colonial Buff Ridgway plate 30; the dorsal fin of the male, Scarlet; and of the female La France Pink, both in Ridgway plate 1; eyes, Turquoise Green, plate 7.

It seems advisable to make a reservation, although it is but a slight one, to the statement that this variety is without traces of external melanophores. A few scattered melanophores of the stipple type may be made out, with the aid of a binocular, upon the back and sides. These cells are all that are left from the normally pigmented form like Stipple, from which it has probably originated. It will be shown (p. 275) that Gold differs from Stipple apparently in only one specific heritable factor, that for 'stipples'. The Gold variety has probably arisen as a recessive mutation. It is hardy and prolific in protected containers, but it is hardly possible that it can survive in the wild habitat, for it is decidedly conspicuous.

This variety has tentatively been named *Platypoecilus maculatus immaculatus* by MYERS (1922b), but the author is doubtful of its subspecific rank.

CULTURAL METHODS[1]

In Mexico, Platypoecilus is found in the tropical fish faunal zone, the fourth and southernmost, the region which lies immediately north of the Isthmus of Tehuantepec. It can not survive our winters without artificial heat. A constant temperature of 24° C has been found suitable

[1] Further details may be found in GORDON (1926d).

for breeding these fishes.[2] This has also been the experience of most of the commercial tropical fish fanciers and of BELLAMY (1924). At this temperature, maturation is hastened and seasonal reproductive habits destroyed, the young being produced every month in the year. It has previously been pointed out that in the wild habitat the fish spawn at the close of the dry season, which in the region of the tropical fish faunal zone extends from April to May.

Viviparity in Platypoecilus presents some difficulties which must be thoroughly appreciated and overcome before successful and accurate work can be carried on. In rearing a school of young fishes for future mating, constant observation is made to detect and isolate those fish which show beginnings of change in the anal fin. Slight thickening of the fore-margin of the anal fin is indicative of rapid modification of that fin into the male intromittent organ. It is by means of this fin that internal fertilization is effected. (The mode of mating in viviparous species of top-minnows has been described by WINGE, 1922 and BELLAMY, 1924). Obviously, differentiating males are not allowed to remain with females because of the likelihood of promiscuous mating. Furthermore, as has been pointed out by GERSCHLER (1914), females once mated, hold sufficient spermatozoa in the folds of their oviducts to serve for the fertilization of many schools of fishes. He records as many as seven schools having been so produced, each at approximately one month's interval. WINGE (1922), reporting on *Lebistes reticulatus*, a viviparous fish of the sub-family Poeciliinae (as is Platypoecilus) says:

> "Some spermatozoa remain in the oviduct where they are able to keep alive for several months, and when the female, a month after fertilization, has cast her young, these spermatozoa can fertilize the eggs which have matured in the meantime."

It has been found advisable, therefore, to use unmated females for all crosses.

One to several females are crossed with a single male. These are placed in a well planted battery jar, eight inches high and six in diameter, which serves for mating purposes. Signs of pregnancy are evidenced by a swelling and distension of the belly in the females. At this time each female is given a separate jar thickly planted with Vallisneria, Elodea and, most important, Riccia. This last plant grows just beneath the surface and

[2] One evening the temperature in the incubator accidently rose from 24°C at 11 P.M. and reached 41°C at 8 A.M. the next morning, thereby killing an important group of fishes. Other battery jars further away from the source of heat had a temperature of 35°C and while the animals were distressed no further losses were experienced. DAVENPORT (1908) does not list any fishes surviving above 40°C.

its dense mesh-like branches offer the newly born fishes an excellent hiding place during their first few hours of utter helplessness. To further prevent cannibalism, which must be carefully guarded against, the parent is given an abundance of food. It is at this particular time that live food, especially Daphnia and Enchytraeus, becomes almost indispensable since quantities may be fed without danger of polluting the water of the aquarium. It further aids in attracting the attention of the parent away from the young fishes. When the young are expelled from their mother's body they straighten out from their previously coiled position and attempt to swim upwards, seeking shelter in the thick vegetation. Within an hour or two their slightly extended yolk sac is entirely absorbed. Most of the members of the newly born school will emerge during the early morning hours, but the entire complement will not usually be expelled until evening. The female is allowed to remain with her young until that time and then removed leaving the progeny in the aquarium. Here they remain for a month or two and then if larger containers are available they are given more room.

In most cases, a count of the offspring is taken about six to eight weeks after birth. At that time they are quite strong and are able to survive a good deal of rough treatment. The period of maturation varies greatly but averages eight months, at which time sex data may be taken.

While it was undesirable to do so, it became necessary occasionally to rear the young to adults in the same small battery jars. As many as ten have been raised in such containers but complete and normal development was slow and difficult to obtain. Because of lack of space only the best specimens were selected after counts were made, and reared to maturity. Only where sex linkage was evident was a special effort made to raise the entire school to maturity. In any given school it is soon evident that individual variation in general vigor and ability to grow is great. Under the best possible conditions a number of young soon drop out and many others never mature. It may be that the present cultures have many inherent lethal factors which inbreeding is bringing out. On the whole, however, barring accidents, when given plenty of room, proper temperature of 24° C and good food, the fishes respond very well.

## EXPERIMENTAL DATA[1]

In the present series of experiments the following strains have been used: Gold, Stippled and Red. They may conveniently be distinguished by these characters:

[1] Summary of data may be found in GORDON (1926c).

| | *Gold* | *Stippled* | *Red* |
|---|---|---|---|
| Melanophores | | | |
| a. Stipples | − | + | + |
| b. Spots | − | − | + |
| Erythrophores | − | − | + |

It is evident from the above that there are three characters which may be studied experimentally, that is, micro-, macro-melanophores and erythrophores. The latter while possible of factorial analysis, have not been extensively studied in these experiments. One or two items of interest that have been worked out with respect to them will be discussed below. The most significant results relate to the melanophores, and the presentation and discussion of data concerning their mode of inheritance follows.

### *The stocks used*

The varieties used in the present experiments proved upon testing to be homozygous for the characters studied. This testing, by matings within the strain, did not precede the varietal crosses, but was run in parallel with them. The pedigree numbers of the original mated pairs and their homozygous progeny are given in table 1. In no case was there a deviation from the parental type in $F_1$, $F_2$ or in $F_3$. In the progeny of Red, there was a slight variation in the number of macro-melanophores exhibited, occasionally more, sometimes less than in the $P_1$, but it is significant that they all possessed spots.

### *The cross of Gold by Red*

Direct: Gold ♀ by Red ♂

The first inter-varietal crosses were made on May 14, 1924. Two homozygous Gold females were mated to homozygous Red males—each cross was run independently and the results recorded separately. These crosses involve factors for *spots*, *stipples*, and *red* (erythrophores)—the Red variety bringing in all the three factors and the Gold variety bringing in none.

The $F_1$ results are summarized from table 2:

$P_1$ GOLD ♀ (Pedigree 10 of school born 1-21-24) NO SPOTS NO STIPPLES
RED ♂ ( " 11 " " " 1-28-24) SPOTTED STIPPLED

| $F_1$ | Spotted Stippled | Non-spotted Stippled |
|---|---|---|
| Observed | 118 | 1 |
| *Expected* | *119* | *0* |

It is quite apparent from the above that the factors for *spotting* and *stippling* are dominant over the absence of such pigmentation. From this one series of experiments alone, one could not differentiate between

the two types of melanophores. It might appear that stipples are merely smaller spots or that spots are merely aggregates of stipples, the factors for the melanophores being one and the same. The process of differentation will become evident in the reciprocal cross, described below.

Limitation of space prevented the rearing of the entire $F_1$ population to maturity. The progeny in the third school of cross, 10–1×11–11 and 10–2×11–12 were all discarded as soon as the count was taken on them (see table 2). Sex ratios were found not to be significant in this series.

Up to the present time the sex ratio for the spotted-stippled class in $F_1$ is as follows: 18 females and 22 males. The latter upon full maturity are red in color but not quite as brilliant as their father, the color being Mars Orange of Ridgway plate 2. The $F_1$ females are similar in color to a homozygous Red female; that is, olivaceous. Since the assumption of red pigment is a secondary sex character of the male it is not surprising that color differences are evidenced. The factor for *red* color, then, is dominant over *non-red*.

The aberrant $F_1$ non-spotted stippled fish appeared singly in a total of 119 individuals. It was reared with great care and, when apparently a mature female, was mated to an $F_1$ male (pedigree 15-11) of known fertility but no progeny were ever produced. It died (unexpectedly) in its eleventh month—unfortunately the internal organs spoiled too rapidly for purposes of further study. A plausible explanation is offered below for its occurrence.

Reciprocal: Red ♀ by Gold ♂

The reciprocal crosses were also set up on May 14, 1924. Two homozygous Red females were mated to one Gold male, the progeny of each female being recorded separately. (Only one Gold male was available at the time). The results from these two crosses and two others made later (for details see table 2) are as follows:

| $P_1$-1 RED ♀ (Pedigree 11 of school born 1-28-24) SPOTTED STIPPLED<br>GOLD ♂ ( " 10 " " " 1-21-24) NO SPOTS NO STIPPLES | | |
|---|---|---|
| $F_1$ | Spotted Stippled | Non-spotted Stippled |
| Observed | 70 | 86 |
| *Expected* | *78* | *78* |

Here the $F_1$ results point to a clear cut distinction between micro- and macro-melanophores—the latter occurring in a ratio of 1:1 while the former are completely dominant. Morphological studies have confirmed the assumption, first drawn from genetical evidence that stipples and

spots are actually as well as apparently different. (See photomicrographs).

The purity of the Red might be brought to question—although the stock proved, by test, to be pure. Ratios of 1:1 would be expected if the females were heterozygous for the *spot* factor. If the ratios of the sexes, male to female, become 1:1 in each class, both in the Spotted Stippled and Non-spotted Stippled, it could be assumed that the females (there were four in all, since two more were added later) were heterozyous. But such is not the case and the evidence from sex ratios is conclusive.

Realizing the importance of the ratio exhibited in the young fishes, special effort was made to rear all the progeny of these crosses. The young of pedigree 16 and 17 were therefore sorted out phenotypically, counted, and given more room, about ten being placed in a jar. The school born 12–1–24 being small was kept separate. In this last school there were originally five Spotted Stippled fishes and six Non-spotted Stippled. Of these eight were reared to maturity and the sex ratio has been found as follows:

| PEDIGREE 16 SCHOOL 12–1–24 | SPOTTED STIPPLED | | NON-SPOTTED STIPPLED | |
|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ |
| Observed | 0 | 2 | 6 | 0 |
| *Expected*[1] | *0* | *2* | *6* | *0* |

Due to an unfortunate accident one night all the rest (38) were killed when they were only four months old so that sex ratios were not obtained. (See footnote 2, page 266). As soon as was possible, this reciprocal cross, Red female by Gold male, was repeated. Six months later the first schools appeared under pedigree 36 and 37. Data on sex are as follows:

| PEDIGREE | SPOTTED STIPPLED | | NON-SPOTTED STIPPLED | |
|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ |
| 36 | 0 | 7 | 12 | 0 |
| 37 | 0 | 38 | 33 | 0 |
| Observed total | 0 | 45 | 45 | 0 |
| *Expected*[1] | *0* | *45* | *45* | *0* |

Here, then, is a clear case for sex-linkage, as against that for autosomal heterozygosity in the female. As yet no exceptions have occurred. Mature

[1] Assuming sex linkage.

Spotted Stippled fishes have always turned out to be males and Non-spotted Stippled fishes have remained female-like, many of the latter producing schools of young. The situation with respect to sex-linkage is similar to that found in Abraxas and in poultry, namely, the ZW type.

Males of pedigree No. 16, 36, and 37 tended to become ***reddish*** just as did the males of the direct cross. This is significant since the female parents of 16, 36, and 37 were Dark Olive Buff and the male parents were Deep Colonial Buff, neither showing red. But it will be recalled that *red* is a male secondary sex character and as such is carried by the olive colored female of the Red strain.

An explanation may now be offered for the appearance of the aberrant sterile, Non-spotted Stippled female in the $F_1$ of the direct cross. Non-disjunction in the $P_1$ male of the Z chromosomes carrying the sex-linked factor for *spots* to give a zygote with no Z chromosome would account for the non-spotted condition in this $F_1$ daughter. The factor for *stipples* being carried by a different set of chromosomes was not affected, so that while the female was *non-spotted*, it was nevertheless *stippled*. The chances of obtaining a ZZW or ZZZ individual are very small since comparatively few individuals have been reared to maturity.

### The $F_2$ (Direct cross)

As a result of four matings a total $F_2$ population of 320 individuals was obtained. Summarized from table 3, the results are as follows:

*$P_1$ Gold ♀ × Red ♂ ($F_1$ spotted stippled ♀ × spotted stippled ♂).*

| $F_2$ | | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
|---|---|---|---|---|---|
| Observed | | 175 | 73 | 52 | 20 |
| *Expected (9:3:3:1)* | | *180* | *60* | *60* | *20* |
| Observed | all spotted | 227 | | all non-spotted | 93 |
| *Expected (3:1)* | | *240* | | | *80* |
| Observed | all stippled | 248 | | all non-stippled | 72 |
| *Expected (3:1)* | | *240* | | | *80* |

Further proof of the individuality of the micro-and macro-melanophores is obtained from the $F_2$ data of the direct cross (above), as well as from the $F_1$ data of the reciprocal cross. In the $F_2$, four phenotypes appear, presenting two new types: the Non-spotted Stippled and the Spotted Non-stippled. The four phenotypes in the $F_2$ indicate that two pairs of factors are operating. The 9:3:3:1 ratio observed (it is not a perfect

agreement, to be sure, but significant) points to dihybridism in the $F_1$ parents.

Due to various causes, this group did not produce as many mature specimens as one might have desired. Particularly striking is the low number of adults in the two Non-stippled classes. Whenever stipples are wanting, the animals are gold colored at birth, and are easily recognized by their flashing brightness. In protected containers, of course, they are not subjected to attacks by predators which naturally would seek out the forms most easily seen. Other factors[1] have been responsible for the low vitality of this class, shown by the $F_2$ adult populations reported below. The stippled fishes, whether spotted or not, are olivaceous at birth.

In the $F_2$ population the Non-spotted Stippled type is obtained which is similar to the $F_1$ females of the reciprocal cross. Theoretically not only should these fishes be similarly colored but similarly sexed. As far as data are available, none of the non-spotted animals have transformed into males, all that have grown to maturity are typically female. Sex data, such as are available, are in agreement with the hypothesis that the *stipple* factor is of autosomal origin and the factor for *spot* shows sex-linkage of the ZW type.

The present results of the examination of the sexes[2] for the $F_2$ are:

| | SPOTTED STIPPLED | | NON-SPOTTED STIPPLED | | SPOTTED NON-STIPPLED | | NON-SPOTTED NON-STIPPLED | |
|---|---|---|---|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Observed | 28 | 41 | 32 | 0 | 5 | 6 | 2 | 0 |
| *Expected numbers* | *21* | *42* | *21* | *0* | *7* | *14* | *7* | *0* |
| *Expected ratio* | *3* | *6* | *3* | *0* | *1* | *2* | *1* | *0* |

## The $F_2$ (Reciprocal cross)

*$F_2$ (Reciprocal cross). $P_1$ Red ♀ × Gold ♂; $F_1$ non-spotted stippled ♀ × spotted stippled ♂.*

| $F_2$ | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
|---|---|---|---|---|
| Observed | 76 | 87 | 16 | 29 |
| *Expected (3:3:1:1)* | *78* | *78* | *26* | *26* |
| Observed | all spotted | 92 | all non-spotted | 116 |
| *Expected (1:1)* | | *104* | | *104* |
| Observed | all stippled | 163 | all non-stippled | 45 |
| *Expected (3:1)* | | *156* | | *52* |

[1] Ancyrocephalus, a parasitic flat-worm, has been observed in the gills of many sick fishes.

[2] Further data on the sexes now available would permit the increasing of the values as presented in this table. Similarly, the values given in sex ratio tables, which follow, may now, also, be extended.

The summarized results from table 3 given above, show the method of dihybrid segregation of the $F_2$ progeny in new proportions. The $F_1$ females, being recessive for the *spot* factor, when crossed with $F_1$ males heterozygous for the same factor, should yield a 1:1 ratio. The observed results 92:116 approach this. Since both females and males ued in these crosses were heterozygous for *stippling*, a 3:1 ratio would be expected in the $F_2$ for this factor. The observed results 163:45 tend to support the assumed conditions.

The sexes of the $F_2$ of the reciprocal cross: The animals making up the $F_2$ total, given above, came from two groups of $F_1$ crosses. Those of pedigree 39 and 40 were obtained quite early from some of the few surviving $F_1$ fishes, 16–1, 16–2 and 16–11 of the school born 12–1–24. About two-thirds of these have matured and the table given below shows the result of the sex count. The remaining $F_2$ progeny derived from pedigree 36 and 37 were obtained much later (see table 3) and are too young for one to determine sex.

Data on sexes of the $F_2$ of the reciprocal cross of the pedigree 39 and 40 (the total $F_2$ population of this group being 70) follows:

| | SPOTTED STIPPLED | | NON-SPOTTED STIPPLED | | SPOTTED NON-STIPPLED | | NON-SPOTTED NON-STIPPLED | |
|---|---|---|---|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Observed | 10 | 9 | 9 | 5 | 4 | 4 | 4 | 4 |
| *Expected numbers* | *9* | *9* | *9* | *9* | *3* | *3* | *3* | *3* |
| *Expected ratio* | *3* | *3* | *3* | *3* | *1* | *1* | *1* | *1* |

It will be seen that the $F_2$ ratios obtained above are quite different from those obtained in the direct cross (see page 271). Here every class has equal numbers of the two sexes whereas in the former case there were no males obtained in the two Non-spotted groups.

### *The backcrosses*

With respect to the direct cross, Gold female by Red male, the $F_1$ progeny were subjected to the four possible backcrosses. While the numbers obtained are small, and possibly not admitting of final analysis of the situation, they tend to corroborate the general hypothesis already formulated: the autosomal nature of the *stipple* factor and the sex linked nature of the factor for *spots*.

In backcross A (table 4) an $F_1$ hybrid female heterozygous for *spots* and *stipples* was crossed with a double recessive male, Gold. The summarized results are as follows:

| | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
|---|---|---|---|---|
| Observed | 8 | 17 | 13 | 7 |
| *Expected* (1:1:1:1) | *11* | *11* | *11* | *11* |
| Observed | all spotted | 21 | all non-spotted | 24 |
| *Expected* (1:1) | | *22* | | *22* |
| Observed | all stippled | 25 | all non-stippled | 20 |
| *Expected* (1:1) | | *22* | | *22* |

| | Sex data | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Observed | 0 | 7 | 9 | 0 | 0 | 5 | 1 | 0 |
| *Expected* | *0* | *5* | *5* | *0* | *0* | *5* | *5* | *0* |

The low number of mature animals reared was directly accounted for by the presence of the parasitic gill flatworm. It is seen, however, that all the *Spotted* fish are *males* and the *Non-spotted, females.*

Backcross B is the reciprocal of the above (A). A double recessive female, Gold was crossed with an $F_1$ hybrid male, heterozygous for *spots* and *stipples*. The results are:

| | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
|---|---|---|---|---|
| Observed | 20 | 42 | 24 | 28 |
| *Expected* (1:1:1:1) | *28* | *28* | *28* | *28* |
| Observed | all spotted | 44 | all non-spotted | 70 |
| *Expected* (1:1) | | *57* | | *57* |
| Observed | all stippled | 62 | all non-stippled | 52 |
| *Expected* (1:1) | | *57* | | *57* |

| | Sex data[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Observed | 5 | 4 | 7 | 5 | 4 | 4 | 6 | 5 |
| *Expected* | *5* | *5* | *5* | *5* | *5* | *5* | *5* | *5* |

[1] Sex data taken June 11, 1926, many yet too young. (See footnote to page 272).

The ratios in the above cross are similar to the former for the various class groups; but here the sexes occur in equal proportion in each class.

Backcrosses C and D are interesting when compared. They show the influence of the sex linked factor which appears in a new proportion. In C when an $F_1$ hybrid female is crossed with a homozygous Spotted and Stippled (Red) male all the progeny are, as expected, Spotted and Stippled, (see table 4 C). If, however, the reciprocal of C is made (as in D) a different result is obtained:

| | SPOTTED STIPPLED | NON-SPOTTED STIPPLED |
|---|---|---|
| Observed | 14 | 3 |
| *Expected (3:1)* | *12* | *4* |

Since the female is considered normally heterozygous for Spots (due to sex linkage) and the male is known to be heterozygous for the same factor, the ratio of 3:1 is expected

Unfortunately many of this school died before sexes became differentiated.

### *The cross of Gold by Stipple*

The individuality of *stipples* is conclusively shown in the following series of experiments and the mode of their inheritance is brought out clearly, uncomplicated by the factor for *spots*.

Two homozygous Gold females were mated to two homozygous Stipple males. These crosses were made in duplicate just as in the former series. The $F_1$ results as given in table 5, show all Stippled offspring (64).

In a similar manner, the reciprocal crosses were set up in duplicate, and the totals in the $F_1$ showed similar results: all Stippled fishes (83).

Carried through the next generation the $F_1$ hybrids of both the direct and reciprocal crosses produced a total of 105 $F_2$ fishes, up to the present time. These form a ratio approaching 3:1 (for details see table 5.)

A case of simple Mendelian inheritance is illustrated above, one factor pair being contrasted; *stipples* are completely dominant over the *lack of stipples*. As the frequencies will indicate, the sexes of the $F_1$ occurred approximately in the proportion of 1:1, the totals being 28 females to 32 males.

## GENERAL DISCUSSION

The experiments reported here, were not undertaken with the purpose of checking up on work already done. The fact that Platypoecilus was being studied, elsewhere, was realized only after the present experiments

were well under way. The brief abstract of the work of BELLAMY (1922) first came to notice only after his later publication in GENETICS, November, 1924, referred to it. In the abstract it was stated that the female Platypoecilus was heterogametic for sex.

His data (1922), he claimed, "disagree in several important respects with the findings of AIDA (1921) and WINGE (1922)." The most important respect obviously is the type of sex linkage found in the different species —females of Platypoecilus being heterogametic for sex would naturally fall under the ZW type, while AIDA and WINGE hold to the XY type for Aplocheilus and Lebistes respectively. Notwithstanding the fact that Lebistes is contained in the same sub-family as is Platypoecilus, and all three fishes belong to the order, Cyprinodontes, it must be remembered that these are different species. It cannot be assumed that what applies to Lebistes or to Aplocheilus must necessarily be true of every Cyprinodont.[1] While, heretofore, sex linkage types have been confined to no lower than ordinal groups—XY for Diptera and ZW for Lepidoptera in insects—as more species of animals are studied it is conceivable that a group lower in scale than a sub-family, might be found to contain species exhibiting different types of sex linkage.

The work reported in this paper has been largely derived from the use of the Gold Platypoecilus—a variety not figured nor described previously in genetical work. Its recessiveness has proved to be a most useful tool in testing dominant factors: *stipples*, *spots* and *red*. It is now being used to test *black*, *crescent*, *blue* and others.

That the female is the heterogametic sex in Platypoecilus is BELLAMY'S hypothesis (1922, 1924) and it is supported by the present investigations. But exception must be made to the following statement: that the factors *W*, *N*, *P*, *R*, (of BELLAMY) constitute a multiple allelomorphic series. Before presenting the evidence, the synonomy of the varietal terms must be set down.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| White | = Stippled, | considered by BELLAMY as a triple recessive. | | | | | |
| Pulchra | = Spotted, | " | " | " | " | dominant for spots only. | |
| Rubra | = Red, | " | " | " | " | " | " red pigment. |
| Nigra | = Black, | " | " | " | " | " | " intense black. |

The factor for *stipples* was not considered by BELLAMY. It is present in all four of his types and could only have been tested with Gold which lacks this character. The variety White merely represents a form recessive to *P*, *R*, and *N* (*spots*, *red* and *black*). The name White is not a fortunate one since the animal is not really white but possesses both micro-

[1] Compare with MORGAN (1926b) p. 205.

melanophores and xanthophores. It is best to reserve the term White for one without color pigment. (Pure white forms have been described in Aplocheilus by TOYAMA 1916, ISHIWARA 1917, and AIDA 1921).

BELLAMY says (p. 523) that "males may be heterozygous for any two of the four characters, *W*, *P*, *R*, or *N;* and the female may carry the determiners for one only." This cannot account for the existence of the *spotted-red* (pulchra-rubra) female. These fishes transmit both *spots* (pulchra) and *red* (rubra) to their sons when mated to a Gold male, a double recessive, (table 2 and page 269).

Very little need be said here of the XY type of sex determination in Lebistes and Aplocheilus. They have been reviewed by CASTLE (1921) and, recently, in greater detail, by CREW (1925) and MORGAN (1926). AIDA'S work (1921) on Aplocheilus confirms the results of an earlier experiment by TOYAMA (1916) with respect to sex linkage. AIDA, however, has accumulated a much stronger body of evidence and his conclusions are significant—some two thousand fishes were reared to maturity, a wonderful cultural project. ISHIWARA (1917) had also worked on Aplocheilus (*Oryzias latepes*) but obtained no indication of sex linkage. This may probably be explained by the fact that his varieties were Yellow-black, (*YB*); Yellow, (*Yb*); Blue, (*yB*); and White, (*yb*); whereas AIDA worked with the factor *R* for red pigment in addition to those above —and *R* the factor for red pigment is the only one that shows sex linkage.[1]

It appears, then, that within the same order, Cyprinodontes, and within the same sub-family, Poeciliinae, different species exhibit the two types of sex linkage. The evidence is primarily genetical. For a more complete solution of the problem, it will be interesting and important to have cytology contribute its share of information.

## SUMMARY

1. *Platypoecilus maculatus* is an extremely variable species in its native habitat. It was first introduced to the aquarists in 1907. By selective methods true breeding types have been isolated.

2. The varieties, Gold, Stippled and Red have been used in the present experiments.

3. The above varieties owe their individuality to the presence or absence of the various color pigments:

A. Erythrophores, red pigment cells, are controlled by the dominant factor *R*, found in the variety, Red.

[1] ISHIMARA cites ISHIKAWA (1913) as having crossed black×yellow to get black $F_1$ and three blacks to one yellow in $F_2$. No sex linkage was evidenced.

B. Melanophores, of which there are two types, differing not only genetically but morphologically: (*a*). macro-melanophores, or spots, controlled by the dominant factor $S_p$ found in the variety, Red. (*b*). micro-melanophores, or stipples controlled by the dominant factor $S_t$ found in the varieties, Red and Stipple.

The variety Gold is recessive for $R$, $S_p$, and $S_t$.

4. $S_p$ is sex linked; $S_t$ is borne by an autosome.

5. The female is heterozygous and the male is homozygous for sex.

6. Platypoecilus has the ZW type of sex inheritance, Lebistes has the XY type—both belong to the sub-family, Poeciliinae, of the order Cyprinodontes.

7. The native habitat and the taxonomy of Platypoecilus are discussed; the cultural methods which were employed in this study are given elsewhere.

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TABLE 1

*Pedigrees of homozygous stocks.*

| $P_1$ | $F_1$ | | | $F_2$ | | | $F_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pedigree Number | Total | How crossed | Pedigree Number | Total | How crossed | Pedigree Number | Total | Grand Totals |
| Gold | 10 | 30 | 10–3×10–12 | 18 | 40 | 18–2×18–12 | 35 | 21 | 91 |
| Red | 11 | 33 | 11–3×11–13 | 19 | 17 | 19–1×19–11 | 26 | 22 | 72 |
| Stipple | 12 | 51 | 12–3×12–13 | 24 | 19 | | | | 70 |

Gold and Stipple stocks obtained from Mr. WAGNER, New York City; Red from Mr. RABENAU Brooklyn, New York.

TABLE 2a

*The direct cross (Gold ♀, non-spotted, non-stippled × Red ♂, spotted, stippled).*

| $P_1$ | | | $F_1$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| GOLD ♀ PEDIGREE | × | RED ♂ PEDIGREE | PEDIGREE | SCHOOLS | | | SPOTTED STIPPLED | NON-SPOTTED STIPPLED |
| 10–1 | | 11–11 | 14 | 8 | 16 | 24 | 18 | 0 |
| | | | | 10 | 28 | 24 | 30 | 0 |
| | | | | 12 | 1 | 24 | 20 | 0 |
| 10–2 | | 11–12 | 15 | 8 | 20 | 24 | 17 | 0 |
| | | | | 10 | 28 | 24 | 13 | 1 |
| | | | | 12 | 1 | 24 | 20 | 0 |
| | | | | | | | 118 | 1 |

TABLE 2b

*The reciprocal cross (Red ♀, spotted, stippled, × Gold ♂, non-spotted, non-stippled).*

| $P_1$ | | $F_1$ | | | | | |
|---|---|---|---|---|---|---|---|
| RED ♀ PEDIGREE | × GOLD ♂ PEDIGREE | PEDIGREE | SCHOOLS | | | SPOTTED STIPPLED | NON-SPOTTED STIPPLED |
| 11–1 | 10–11 | 16 | 10 | 29 | 24 | 11 | 16 |
| | | | 12 | 1 | 24 | 5 | 6 |
| 11–2 | 10–11 | 17 | 10 | 7 | 24 | 6 | 12 |
| 19–3 | 18–12 | 36 | 4 | 19 | 25 | 7 | 14 |
| 19–4 | 18–13 | 37 | 4 | 19 | 25 | 15 | 13 |
| | | | 5 | 28 | 25 | 17 | 17 |
| | | | 7 | 3 | 25 | 9 | 8 |
| | | | | | | 70 | 86 |

TABLE 3a

*The $F_2$ of the direct cross ($P_1$, Gold ♀ × Red ♂, table 2a).*

| $F_1$ | | $F_2$ | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SPOTTED STIPPLED PEDIGREE ♀ | × SPOTTED STIPPLED PEDIGREE ♂ | PEDIGREE | SCHOOLS | | | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
| 14–1 | 15–11 | 28 | 2 | 25 | 25 | 16 | 4 | 11 | 1 |
| | | | 4 | 26 | 25 | 15 | 7 | 3 | 1 |
| 14–2 | 15–12 | 29 | 3 | 15 | 25 | 15 | 7 | 8 | 4 |
| | | | 5 | 1 | 25 | 13 | 4 | 1 | 0 |
| 14–3 | 15–13 | 30 | 3 | 20 | 25 | 20 | 3 | 7 | 2 |
| | | | 5 | 15 | 25 | 14 | 12 | 2 | 1 |
| 15–1 | 16–11 | 38 | 7 | 2 | 25 | 18 | 4 | 2 | 0 |
| | | | 11 | 9 | 25 | 24 | 8 | 5 | 2 |
| | | | 12 | 19 | 25 | 40 | 24 | 13 | 9 |
| | | | | | | 175 | 73 | 52 | 20 |

TABLE 3b

*The $F_2$ of the reciprocal cross, ($P_1$, Red ♀ × Gold ♂, table 2b).*

| F-1 | | F2 | | | | | |
|---|---|---|---|---|---|---|---|
| NON-SPOTTED STIPPLED PEDIGREE ♀ | SPOTTED STIPPLED PEDIGREE ♂ | PEDIGREE | SCHOOLS | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
| 16–1 | 16–12 | 39 | 8 18 25 | 6 | 4 | 5 | 0 |
| 16–2 | 16–12 | 40 | 9 20 25 | 2 | 8 | 2 | 2 |
| | | | 1 10 26 | 16 | 10 | 4 | 11 |
| 37–1 | 36–11 | 53 | 6 29 26 | 14 | 21 | 1 | 7 |
| | | | 7 27 26 | 18 | 17 | 0 | 3 |
| 37–2 | 36–11 | 54 | 7 14 26 | 6 | 13 | 0 | 3 |
| | | | 8 11 26 | 14 | 14 | 4 | 3 |
| | | | | 76 | 87 | 16 | 29 |

TABLE 4

*The backcrosses.*

| PEDIGREE ♀ | PEDIGREE ♂ | NEW PEDIGREE | NUMBER OF SCHOOLS | SPOTTED STIPPLED | NON-SPOTTED STIPPLED | SPOTTED NON-STIPPLED | NON-SPOTTED NON-STIPPLED |
|---|---|---|---|---|---|---|---|
| Cross A | | | | | | | |
| $F_1$ × Gold | | | | | | | |
| 14–5 | 18–11 | 33 | 2 | 8 | 17 | 13 | 7 |
| Cross B | | | | | | | |
| Gold × $F_1$ | | | | | | | |
| 18–1 | 14–12 | 34 | 1 | 2 | 3 | 3 | 3 |
| 35–1 | 16–11 | 44 | 4 | 18 | 39 | 21 | 25 |
| | | | Total | 20 | 42 | 24 | 28 |
| Cross C | | | | | | | |
| $F_1$ × Red | | | | | | | |
| 14–4 | 19–11 | 31 | 2 | 50 | 0 | 0 | 0 |
| Cross D | | | | | | | |
| Red × $F_1$ | | | | | | | |
| 19–2 | 14–11 | 32 | 1 | 14 | 3 | 0 | 0 |

TABLE 5a

*The cross of Gold by Stipple (Gold ♀, non-stippled × Stipple ♂, stippled).*

| $P_1$ | | $F_1$ | | | | | |
|---|---|---|---|---|---|---|---|
| GOLD PEDIGREE ♀ | × STIPPLE PEDIGREE ♂ | PEDIGREE | STIPPLED | | | STIPPLED | NON-STIPPLED |
| 10–3 | 12–11 | 20 | 3 | 1 | 25 | 25 | 0 |
| 10–4 | 12–12 | 21 | 3 | 1 | 25 | 21 | 0 |
| | | | 4 | 15 | 25 | 18 | 0 |
| | | | | | | 64 | 0 |

TABLE 5b

*The reciprocal cross (Stipple ♀, stippled × Gold ♂, non-stippled).*

| $P_1$ | | $F_1$ | | | | | |
|---|---|---|---|---|---|---|---|
| STIPPLE PEDIGREE ♀ | × GOLD PEDIGREE ♂ | PEDIGREE | SCHOOLS | | | STIPPLED | NON-STIPPLED |
| 12–1 | 10–12 | 22 | 1 | 1 | 25 | 18 | 0 |
| | | | 1 | 17 | 25 | 12 | 0 |
| | | | 3 | 1 | 25 | 25 | 0 |
| 12–2 | 10–13 | 23 | 1 | 8 | 25 | 28 | 0 |
| | | | | | | 83 | 0 |

TABLE 5c

*$F_1$ inbred to give $F_2$.*

| $F_1$ | | $F_2$ | | | | | |
|---|---|---|---|---|---|---|---|
| PEDIGREE ♀ | PEDIGREE ♂ | PEDIGREE | SCHOOLS | | | STIPPLED | NON-STIPPLED |
| 20–1 | 20–11 | 45 | 12 | 27 | 25 | 12 | 2 |
| | | | 1 | 23 | 26 | 24 | 4 |
| 20–2 | 20–11 | 46 | 12 | 13 | 25 | 15 | 5 |
| 22–1 | 20–11 | 47 | 1 | 16 | 26 | 8 | 2 |
| | | | 12 | 12 | 25 | 11 | 3 |
| 22–2 | 20–11 | 48 | 1 | 23 | 26 | 15 | 4 |
| | | | | | | 85 | 20 |

# SOME ATTEMPTS TO MODIFY THE GERM PLASM OF *PHASEOLUS VULGARIS*[1]

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TABLE OF CONTENTS

| | PAGE |
|---|---|
| INTRODUCTION | 284 |
| Description of materials and methods | 285 |
| Inheritance of modifications | 287 |
| DISCUSSION | 293 |
| LITERATURE CITED | 294 |

## INTRODUCTION

Several investigators have called attention to various chromatin and other nuclear abnormalities in cells of *Vicia faba* with the application of chloral hydrate. However, the extent to which heritable variations may be produced by such treatment has not been reported as far as the writer could learn. This should be of considerable scientific value. Further experiments, therefore, on attempts to modify the germ plasm with chloral hydrate applications and then to determine to what extent heritable variations may thus be induced is fully warranted.

NĚMEC (1903) calls attention to the occurrence of true heterotypic divisions of treated root tips. SAKAMURA (1920) states that various chromatin abnormalities may be produced and KEMP (1910) points out that any of the following may take place: An arrested movement of the split chromosomes to the opposite poles; the refusion of split chromosomes and the formation of a single large nucleus; double nucleated cells; amoeboid or "bridge" nuclei; and abnormally small or abnormally large nuclei. KEMP also found what seemed to look like heterotypic divisions in plant tissue which ordinarily does not produce germ cells. He states that these are merely the results of some physico-chemical effect of the chloral hydrate and are not true heterotypic divisions. Experiments with this drug are described by HOTTES (1924). He points out the striking effects upon the chromatin content of cells treated with either of these chemicals. His cytological studies revealed some marked departures in

[1] Abstract of thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agronomy in the Graduate School of the UNIVERSITY OF ILLINOIS, 1926.

the migration of the chromosomes and particularly in the arrangement and structure of the chromatin material. Cells passing into the resting stage after having been treated with these drugs showed chromatin more or less irregularly broken up often into more than a dozen separate parts, each one of which rounded off into separate nuclei devoid of spindle fibres. At other times unequal division and subsequent refusion resulted in the formation of either abnormally small or abnormally large nuclei.

## DESCRIPTION OF MATERIAL AND METHODS

The investigations reported in this paper were made with a strain of Navy bean, *Phaseolus vulgaris* which had been selected for genetic purity. The Navy bean was chosen, first, because of its cosmopolitan nature and wide adaptability, second, because of its high degree of constant uniformity and its comparative freedom of departures from type, and third, because it is self-fertilized and only rarely, if at all, cross-fertilized. A carefully selected strain of Navy bean was therefore thought to be good material to work with from the standpoint of studying any heritable variations that might be induced through artificial means.

Since HARRIS, et al (1916, 1920) show that abnormalities occur in the Navy bean to the extent of at least one in fifty-seven and that a heritable departure from type has been found, it was, therefore, very essential, before subjecting a strain of the Navy beans to any special treatment, to determine the nature and extent of the departures from type which might occur without treatment. Otherwise the occurrence of a modification might have been credited to a treatment when it should have been attributed to something else.

Although variations were found in the selected strains of the Navy bean used for experimentation by the writer, these variations did not quite correspond to those described by HARRIS and his co-workers. Certain departures from type were observed in plantings made at Nebraska, Kentucky, Indiana, and Illinois, covering a period from 1910 to 1924. Out of 194,654 plants grown to maturity and 18,071 through the seedling stage, only 424 showed deviations from type. The deviations found were, therefore, approximately one in five hundred. This is a much lower percentage of deviates than HARRIS and his associates found. This low percentage found by the writer is perhaps due to the fact that the lines were selected for uniformity. The deviates that were found in this selected strain were either fasciations or showed an opposite leaf insertion instead of the normal alternate arrangement. Others had more than two cotyledons. In some instances, types were found with fasciated seed leaves

which inhibited further growth of the plant. In no case, in the progeny of the different deviates described, were deviates found occurring in greater frequency than in the progeny of normal types. Thus these were not heritable variations.

FIGURE 1.—Number 1, a normal untreated plant. Number 2, a plant treated with .75 percent chloral hydrate solution before the plant commenced to vine. Number 3, a plant treated with .75 percent chloral hydrate solution after the plant had vined.

Various concentrations of chloral hydrate were tried at first to find out the maximum concentration that could be used to produce modification without killing the treated seed or growing plant. Seventy-five hundredths of one percent gave the best results. The solution was applied throughout the entire cycle of development from seed to seed in one of the following

ways: (a) Germination of seeds between blotters kept moistened with chloral hydrate solution and then planted and allowed to grow into maturity with or without further treatment with this drug; (b) like (a) except that chloral hydrate solution was added to the base of the growing plant so long as there was no danger of killing it; (c) application of chloral hydrate solution to the base of growing plants in various stages of development from the time that the seedling emerged from the soil through to the production of seed; and (d) provision for the penetration of the solution through and into the axil of the leaf, by means of a fine thread the opposite ends of which were brought in contact with absorbent cotton kept moist with the chloral hydrate solution.

Seeds germinated in chloral hydrate and allowed to grow without further treatment produced at first abnormal growth. Later, however, normal growth developed without the recurrence of any of the initial modified growth. In the end such a plant had lost all of its former abnormal appearance and became normal in appearance. Only by occasional repeated applications of the chloral hydrate solutions to the base of the plant stem could modified growth be maintained (See figure 1, number 2). Treatment confined to certain parts of the plant caused the portion above the point of application to become modified (See figure 1, number 3). If the plant had been treated after it had made a certain amount of normal growth, all subsequent leaf development showed various degrees of modification until further treatment was again discontinued. Then the new growth was of a normal character. The range of leaf variability caused by chloral hydrate treatment is illustrated in figure 2. A comparison of figure 2 with figure 1, number 1 brings out the contrast between the normal trifoliate and the modified leaf shapes.

By way of summary, it may be stated that the modifications caused by chloral hydrate were as follows: (1) Marked constriction and blunting of the radicle; (2) marked constriction of the hypocotyl, stem and petioles, giving the seedling, the plantlet and plant, if the solution was occasionally applied, a very much dwarfed and constricted appearance; (3) a pale yellowish-green greasy appearance and a blistered mosaic pattern very much like that found in plants infected with virus diseases; (4) fasciation of the stem, petioles and leaves; (5) modification of the characteristic tri-foliate leaf symmetry; and (6) the production of various abnormal leaf shapes and sizes (See figure 2).

## INHERITANCE OF MODIFICATIONS

In the first generation of the chloral hydrated progeny, 146 out of 200

plants were aberrant types. Among these, the first leaf whorl was altogether different from the normal trifoliate of the controls. The nature of the modification of the primordial leaf is shown in figure 3. Instead of the normal trifoliate leaf type others were found which varied in shape from single or unifoliate unilobed to five leaved or pentafoliate types. The pentafoliate types are illustrated in number 18 of figure 3. Although modifications from the normal trifoliate type were found in other bean varieties, no modifications, such as have been shown to occur as a result

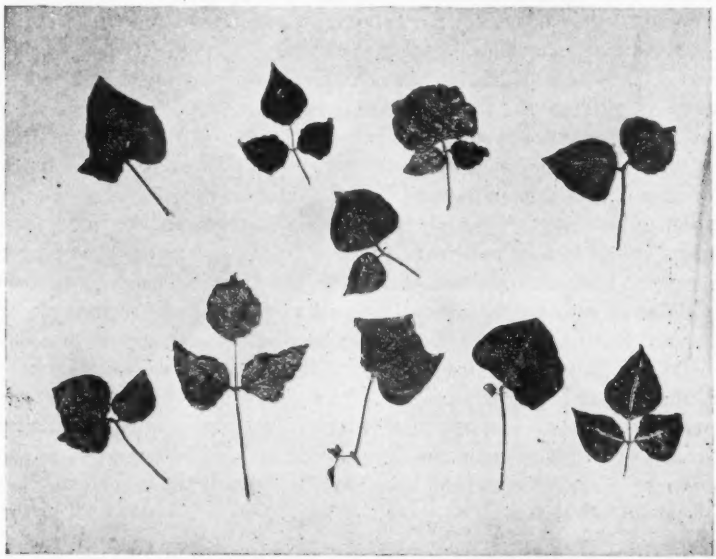

FIGURE 2.—Types of leaves found after plants were treated with .75 percent chloral hydrate solution. Compare with leaves of untreated plant shown in figure 1, number 1.

of initial chloral hydrate treatments, were in any instance found in the strain of Navy bean from which the seed material for treatment was derived. When compared, the type of aberrancy produced by the first generation progeny of the chloral hydrated parents was found to be entirely different from any type departures observed in 194,645 plants grown to maturity and 18,017 seedlings where no treatment was used. Furthermore, the proportion of aberrants to normals in the progeny of the treated parents was 1.37 to 1 as compared with one abnormal to five hundred normals in the first generation of the untreated plants. These

results indicate that chloral hydrate was an effective means of modifying the parent and of causing a high percentage of new aberrant types to be produced.

The most extreme of the 146 modifications found in the first generation, were grown to maturity and seed was harvested from them to make up a series composed of 200 seedlings for the second generation. Likewise, in each succeeding generation, seed was gathered from the most extreme aberrants to provide for a series composed of 200 seedlings for the next

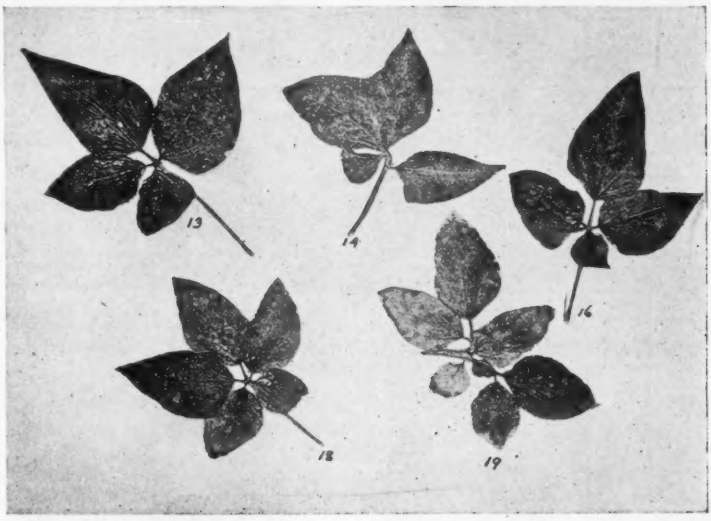


FIGURE 3.—Aberrant leaves found in first generation descendants of a parent initially modified with chloral hydrate. Number 19 is designated as fasciated, number 18 as pentafoliate, and numbers 13 and 14 as quadrifoliate.

generation. (See figures 4 and 5). The number and percentage of aberrants in 200 such seedlings for each generation is shown in table 1.

A decrease in the number of abnormalities is shown up to the fourth generation when a slight increase is noticed. However, the degree of modification found among the 103 plants recorded in the fourth generation was in the aggregate much less than that found among the 94 third generation plants. Thus from the standpoint of total modifying effect, there was a general falling off of aberrancy in each generation. To determine this, observations were made of the amount of variation carried

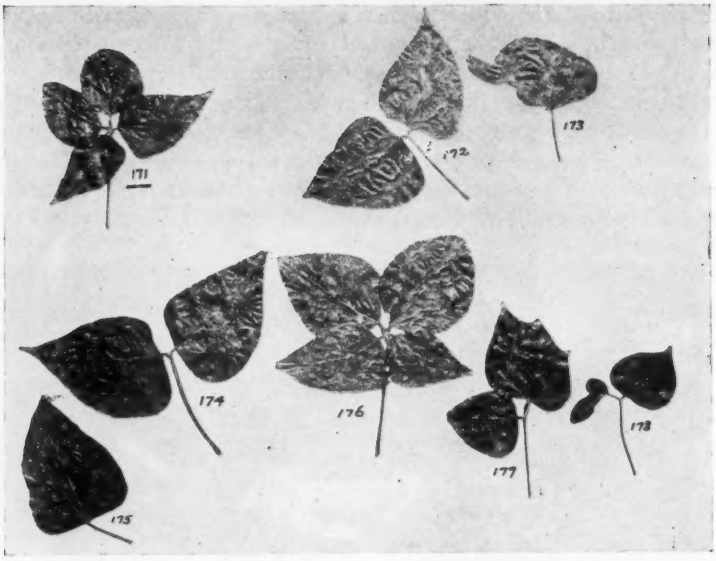


FIGURE 4.—Aberrant leaves found in second generation descendants of a parent initially modified by chloral hydrate. This is the first generation of aberrancy.

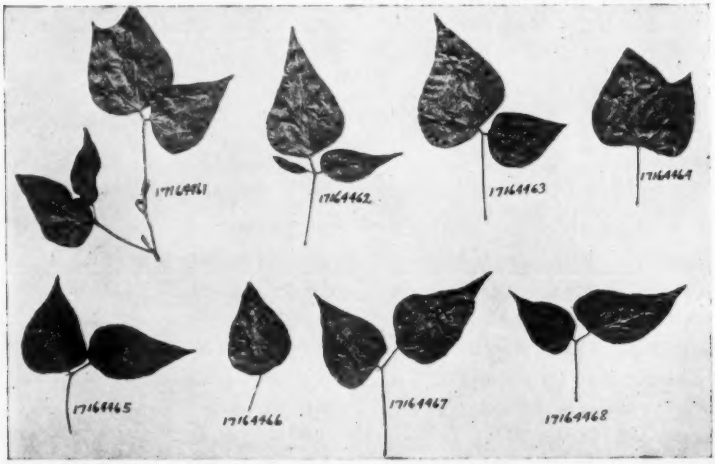


FIGURE 5.—Aberrant leaves found in the seventh generation descendants of a parent initially modified by chloral hydrate. This is the sixth generation of aberrancy.

over into succeeding generations, when certain types of aberrants were selected as parents (See table 2). Two pentafoliate types 76 A (14) and 84 B (3) produced 67.6 and 41.1 percent of aberrancy respectively. However, although 76 A (14) produced a higher percent of aberrant types than 84 B (3), the latter produced 13.5 percent quadrifoliate aberrants while 76 A (14) produced only 9.6 percent. The progenies of two quadrifoliate and two unifoliate aberrations behaved somewhat similarly in this respect to the pentafoliate types. The two quadrifoliate types produced respectively 64.9 per cent aberrancy with 3.2 percent quadrifoliates, and 70.6 percent aberrancy with 0.0 percent quadrifoliates. The differences between the unifoliates was 1.9 percent aberrancy and 0.0 aberrancy. From these data it would seem that although the same types of modifications produce different types of aberrants in their progeny, the aberrancy from a quantitative standpoint is about the same.

Judging from the decrease of modifications in each succeeding generation, it would appear that the modifications produced as a result of the initial chloral hydrate treatment were not due to any alteration of chromatin matter. This was verified in crosses made by pollinating normal plants with the pollen of plants treated and modified by chloral hydrate. The progeny of such a cross showed no variation from normal plants nor did the backcrosses of this progeny with chloral hydrated plants. Thus the persistency of the modifications into the sixth generation must have been due to some cause other than an alteration of the chromatin material.

TABLE 1

*Number and percent of modifications in each generation due to an initial treatment with .75 percent choral hydrate.*

| GENERATION | NORMALS | | ABNORMALS | | TOTAL |
|---|---|---|---|---|---|
| Number | Number | Percent | Number | Percent | |
| 1 | 54 | 27 | 146 | 73 | 200 |
| 2 | 66 | 33 | 134 | 67 | " |
| 3 | 106 | 53 | 94 | 47 | " |
| 4 | 97 | 48.5 | 103 | 51.5 | " |
| 5 | 184 | 92 | 16 | 8 | " |
| 6 | 192 | 96 | 8 | 4 | " |
| 7 | 200 | 100 | 0 | 0 | " |

It seems that there are three ways in which the carry-over effect might have been brought about, namely: (1) The mechanical or actual carry-over of some of the chloral hydrate in either the cytoplasm of the egg or in the tissue of the seed or in both; (2) the carry-over effect of an altered seed tissue that had reacted to chloral hydrate while developing

on the treated parent plant, and (3) the carry-over effect of an altered cytoplasm of the egg cell. In view of the fact that seeds germinated in .75 percent chloral hydrate lost their aberrant nature unless treatment was kept up from time to time, the amount of chloral hydrate that could be carried over mechanically would be insufficient to maintain the degree and persistency of aberrancy secured. For this reason it would seem very

TABLE 2

*Kinds of aberrancy in the progeny of selected aberrant parent types from grandparents initially modified by chloral hydrate.*

| PARENTAGE | | NUMBER OF SEEDLINGS | CLASSIFICATION OF ABERRANTS | | | | | | | | | | NUMBER OF ABERRANTS | PERCENT ABERRANTS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Quadrifoliate | | Unifoliate | | | | | | Miscellaneous | | | |
| | | | | | Trilobed | | Bilobed | | Unilobed | | | | | |
| Type | Label Number | | No. | Percent | No. | Percent | No. | Percent | No. | Percent | No. | Percent | | |
| Pentafoliate | 76A (14) | 83 | 8 | 9.6 | 11 | 14.2 | 7 | 8.2 | 8 | 9.6 | 22 | 2.7 | 57 | 67.6 |
| | 84B (3) | 102 | 14 | 13.5 | 3 | 2.9 | 3 | 2.9 | 2 | 1.9 | 14 | 13.5 | 42 | 41 |
| Quadrifoliate | 76A (17) | 91 | 3 | 3.2 | 16 | 17.5 | 3 | 3.2 | 11 | 12.1 | 26 | 28.5 | 59 | 64.9 |
| | 84B (4) | 109 | 0 | 0 | 11 | 10.1 | 16 | 14.6 | 12 | 11 | 37 | 33.9 | 76 | 70.6 |
| Unifoliate | 76A (21) | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | .9 | 1 | .9 | 2 | 1.9 |
| | 85B (11) | 83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

unlikely that the seed, much less the very small amount of cytoplasm in the egg cell, could carry over the amount of chloral hydrate that maintains the aberrancy. There is some possibility that the seed tissue while developing on the treated parent may have reacted and that a changed seed tissue was carried over. But such a carry-over could last only one generation. For a more extended effect lasting into several succeeding progenies a more deep-seated reaction must have taken place. Such an extended effect seems most likely to have occurred as a result of an actual modification of the egg cytoplasm and its inclusions with the resultant carry-over from one generation to another of an altered cytoplasm. With time, the altered cytoplasm became gradually restored to its normal state and as

a consequence aberrancy fell off correspondingly. Of the three explanations the last seems the most plausible.

## DISCUSSION

It has been shown in the experiments herein described that plant material which had been carefully selected and grown for genetic purity, when treated initially with .75 percent chloral solution, not only produced some very marked modifications, but produced aberrant types which persisted through six generations. Just how the chloral hydrate solution brought about the effects in the parent which caused it to be the progenitor of a very aberrant progeny, is still unaccounted for by the writer. That these aberrancies are not heritable changes has been shown (1) by a diminution of aberrant types in each succeeding generation; (2) because normal plants pollinated by chloral hydrated plants or by aberrant types selected from the progeny of parents that had been treated and modified by chloral hydrate, produced no aberrant types, and (3) because the hybrids of normals × chloral hydrate modifications back-crossed with chloral hydrate types showed no modifications. The persistence of the aberrant types were, therefore, not due to a change in chromatin material. If this were so, obviously the aberrancy would have been handed down by the generative nucleus of the pollen grain also. This was not the case, and, therefore, the transmission must have been entirely by means of the egg.

In so far as it has been shown that the chromatin material of the male nucleus does not transmit a factor for aberrant leaf shapes, it could be concluded that the chromatin material is not sufficiently altered. Aside from the chromatin material of the egg, the only material left to carry over the effects, is the cytoplasm and of course its inclusions. An explanation of carry-over effects made upon such a basis may, therefore, be considered in this connection.

There are some reasonable arguments to be made in favor of the carry-over of certain properties by means of the cytoplasm in the egg. It is quite possible that the cytoplasm of the egg had been caused to react in a peculiar manner to the effects of the chloral hydrate solution and thus had become altered. An altered cytoplasm of the egg may from then on have been carried over in the subsequent cell divisions. As long as such modified cytoplasm was handed down, aberrancies were produced accordingly.

The part that cytoplasm plays in regard to the transmission and the initiation or cause of certain developmental processes, is brought out by

MORGAN (1924). It has been pointed out by him, that such relations as right and left, anterior and posterior and the spatial parts of the embryo in relation to the early established plans of symmetry and localization of organs along axial lines, seem to be laid down in the beginning of development. Thus the altered symmetry of leaves in the progeny of the bean plants initially modified by treatment with the chloral hydrate solution may be accounted for on the basis that the cytoplasm was altered by a reaction to this solution. MORGAN states further that it is surprising that so few instances have been found that are referable to the cytoplasm since there is an ever increasing body of information relating to cytoplasmic inclusions.

It appears to the writer that these chloral hydrate results have a certain significance in a study of the after effects of exposure to various conditions of disease, insect injuries, toxicity and too abrupt changes in the temperature as well as many other environmental changes. Such conditions could bring on an initial modification of the cytoplasm. The carry-over of a modified cytoplasm would then be the cause of aberrant types even when the initial cause which brought about a change in the cytoplasm is absent. In this way the after effects of certain factors causing the initial modification may persist for several generations. This may be an explanation for the origin and then the gradual disappearance of some of the so-called bud sports and somatic mutations.

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# A CASE OF NON-DISJUNCTION IN THE MOUSE[1]

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Occasionally certain phenomena of inheritance occur which cannot be explained on the basis of Mendelism. An exception of this sort was reported by BRIDGES (1913) and explained on the hypothesis that rarely two homologous chromosomes do not disjoin following synapsis.

Further observations concerning this phenomenon of non-disjunction in Drosophila have been reported by BRIDGES (1913, 1914, 1916, 1925) and L. V. MORGAN (1922). Conclusive proof of the occurrence of the process has been produced both from a genetical and from a cytological standpoint. In plants also, BLAKESLEE (1922) and BELLING and BLAKESLEE (1922) have observed a similar phenomenon in Datura. They also find cytological proof of non-disjunction in the chromosomes of the cells, producing triploid and tetraploid groups.

It is in the inheritance of sex-linked characters in animals, however, that the "exceptions" to Mendelian inheritance have been mostly noted; and it is in these cases that genetical proof of non-disjunction is most easily established. Non-disjunction of autosomes is not so easily established from a genetical standpoint on account of the presence of paired chromosomes in both male and female.

However, genetical and cytological proof of non-disjunction of autosomes has been furnished by BRIDGES (1921) in the fourth chromosome of Drosophila, and by R. R. GATES and N. THOMAS (1914) in Oenothera and by others. Other cases of abnormal inheritance are probably also explainable on this hypothesis.

The following instance of non-Mendelian inheritance may be explained on the hypothesis of non-disjunction, or else of deficiency of a part or whole of an autosome. Genetically, in the absence of linked characters these phenomena would be indistinguishable, and would yield similar results when subjected to tests of inheritance.

In an attempt to find the possible linkage relationship of waltzing with other genetic characters in mice, an initial cross between a pink-eyed,

[1] A preliminary report of this paper was read at the meeting of the American Society of Zoologists at New Haven, Dec. 19, 1925.

dilute, brown mouse and a piebald waltzer was made. The latter was the male parent and was of the pure Japanese waltzing strain, brother-sister mated for nine generations and inbred before this for many years. The former was an inbred strain, brother-sister mated for seven generations and was developed through a mating of Doctor LITTLE'S race of dilute browns, with a pink-eyed male from Doctor STRONG, which itself had been produced by an out-cross of the above race of dilute browns. The waltzing character has never appeared, so far as known, in the ancestry of the normal parent. Of this mating, several hundred individuals were produced all of which were perfectly normal, according to expectation, both with respect to the waltzing character and all other genetic characters involved. In one litter, however, of seven individuals, of which three were males and four females, female No. 1913, was a waltzer. With respect to all of its other characteristics it appeared normal, as were all of its sibs.

As this waltzing individual appeared as an exception in an $F_1$ generation, she will be called the $F_1$ waltzer.

Her mother had previously given birth to two litters containing a total of nine individuals, all of which were normal according to expectation. Following this litter, likewise, the mother gave birth to six young, in one litter, all of which were normal. It therefore seems improbable that there could have been any unusual genetic condition present in the mother, and, as will be shown later, any unusual condition in the father would not have resulted in the production of a waltzer in an $F_1$ generation.

As waltzing is completely recessive to normal, and as the writer has never observed any intermediate condition, nor any mice in which the waltzing character could possibly be mistaken for normal, this instance of a waltzing mouse in an $F_1$ generation is exceptional and requires explanation.

Several possible explanations might be given for a condition of this kind, such as, (1), a pathogenic or pathological abnormality, as undoubtedly occurred in MORGAN'S (1911) artificial waltzers, in which case the genetic formula of the $F_1$ waltzer would be that of a normal heterozygote, *Vv*; or, (2), it might be a mutation to waltzing of the normal allelomorph of the waltzing gene, giving a genetic formula of *vv*; or, (3), a new dominant waltzing mutation producing a mouse of the genetic formula *VvXX* or *VvXx*; or, again, (4), it might be a case of non-disjunction of the two chromosomes carrying the factor for normal gait resulting in the zygote *vo*; or, (5), finally, it might be a deficiency (loss) of the gene normal with or without loss of other portions of the autosome in which it lies. The last two possibilities would be indistinguishable in their

genetic behavior, so long as there is no known mutation linked with the waltzing factor. The genetic formula in either of the last two cases would be *vo*.

Perhaps the most obvious explanation that might be offered by those skeptical of the case would be that there was a mix-up in the family and that the fence line between two pens was not entirely secure, and that the waltzing individual did not belong to the litter in which it was found. Such a condition could hardly have existed, particularly under the conditions, as practically my entire stock was given over to the raising of this particular $F_1$ generation and the mice were cared for exclusively by the writer.

No other *male* could have produced this mouse, since by any other male, the young should still all be normal. The only possible question is concerning the *mother*.

Fortunately, this individual, if of the supposed mating, would be heterozygous for four distinct genetic characters, exclusive of waltzing, so that a test breeding with an ultimate recessive would reveal her correct constitution as checked by four pairs of characters. She was accordingly bred to a pink-eyed, brown, piebald waltzer that was heterozygous for dilution. The offspring from this mating should, theoretically, produce fifty percent each of pink-eyed, brown, and piebald, and twenty-five percent of dilute. The first litter obtained from this mating gave five individuals Nos. 3061–3065 inclusive, of which three were pink-eyed, three brown, two piebald, and one dilute, showing the closest possible agreement with expectation and thus establishing her genetic constitution. All the young produced from this mating were waltzers.

Incidentally, it might also be noted that this individual possessed the usual hybrid vigor observed in all of the individuals produced by this particular cross (Japanese fancy), GATES (1926). She lived to be 26 months old and produced more litters than is normal for waltzers. She finally died from the effects of cancer, which is not uncommon in the Harvard strain of Japanese waltzers, and susceptibility to which character is transmitted to the $F_1$ generation, TYZZER (1909), and LITTLE and TYZZER (1916). Waltzers are, in general, shorter lived than the common fancy mice, seldom reproducing beyond the age of twelve months. This hybrid vigor together with the tested genetic characters of the $F_1$ waltzer seems to indicate that there could be absolutely no question as to her origin from the same cross as her sibs.

Of all the possibilities, that of a pathogenic or pathological individual was eliminated in the first mating, for if she were a waltzer due to some

physiological cause, her *genetic* constitution would be that of a heterozygote and her young by a waltzing male would be half of them waltzers, half non-waltzers. To test further this possibility with greater numbers, her second mating was with a pure Japanese waltzing male, and she produced from this mating six individuals, *all* of which were waltzers. It is hardly possible that a heterozygous individual bred to a homozygous recessive would produce eleven recessives consecutively, without a single dominant, when expectation is half and half. This is a deviation of $5.5 \pm 1.12$ from expectation and the odds against such an occurrence by chance 1051 to 1. The possibility therefore of the waltzing condition being pathogenic or pathological was eliminated by these first two matings.

There remained four possible explanations for this phenomenon, any of which would be compatible with the results of her first two matings. These are, (1), a dominant mutation, (2), a mutation in the chromosome carrying the normal factor, (3), non-disjunction, and (4), deficiency. Further matings were therefore made in an attempt to analyze these possibilities. The $F_1$ waltzer was therefore next mated to a normal male, No. 2907, from Dr. LITTLE's stock of dilute browns. This race has been very closely inbred by him for years and this particular male is from an $F_8$ generation of the author's stock derived from this source. *These dilute browns, so far as known, have never produced a waltzing individual.*

The result of this mating was a litter of six, Nos. 4149–4154 inclusive, two males and four females, all normal in every respect. A second mating was made with this male and two young were obtained, both males and both normal. The last mating of the $F_1$ waltzer was made with another dilute brown male of the same strain, and produced five young, all normal. Thus thirteen normal young and no waltzers should be sufficient proof that the waltzing character was *not* a dominant mutation, either in a homozygous or heterozygous condition.

By the process of elimination, three possibilities are still tenable, and proof for those would rest on the breeding behavior of the offspring.

The next mating of the $F_1$ waltzer was with her own son, a waltzer, No. 3065, one of the males in the first litter produced. This mating was made to test the genetic constitution of both the $F_1$ waltzer and her son. According to the remaining hypotheses about the $F_1$ waltzer, this son could be a normal homozygous waltzer, *vv*; or, if this is a case of non-disjunction, he might be similar to his mother, a simplex waltzer, *vo*. This mating between mother and son produced four young, Nos. 4696–4699 inclusive, two females and two males, all of which were waltzers.

It was hoped that if this male were of a constitution *vo*, nulliplex forms *oo* might be obtained, but such were not observed.

The mating was repeated and the $F_1$ waltzer produced, this time, six young, all of which were waltzers. This test was then abandoned as the only thing to be learned from it was either that the original female was a mutation of the gene normal, or that the male used was a homozygous waltzer, or that nulliplex individuals are not produced.

The seventh mating of the $F_1$ waltzer was with her tested son, No. 4153. This male had shown himself to have a genetic constitution of *Vo*. Mated to the $F_1$ waltzer, *vo*, four types of young are expected in equal numbers, *Vv*, *Vo*, *vo*, *oo*, of which the *vo* individuals will be waltzers. From this mating seven young were produced, five normal and two waltzers. It was hoped that this mating might also give a clue as to what becomes of the *oo* individuals, but from the results gotten, no conclusion can be drawn.

While the $F_1$ waltzer showed hybrid vigor to a marked degree, her offspring, particularly the waltzers, seemed to be a good deal less viable, and only a few survived to full maturity. The normal individuals, however, appeared to be quite viable, and lived and grew normally. Of the six individuals in her third litter five survived to maturity, two males and three females. These individuals were brother-sister mated to test their genetic constitution and also to test the remaining possibilities, namely, mutation and non-disjunction.

If, now, the $F_1$ waltzer is due to mutation of the gene normal, *all* of her offspring by a normal male would be heterozygous for waltzing and should produce twenty-five percent waltzers in an $F_2$ generation. Actually, as will be shown later, and can be seen from the table, some of her young by this mating do *not* produce waltzers. The possibility therefore of a mutation of the normal gene is eliminated.

If this is a case of non-disjunction, the hypothesis to account for a waltzer in the $F_1$ generation would imply that the two autosomes carrying the gene normal did not only not disjoin, but both passed out into the polar body of the egg leaving the egg nulliploid rather than haploid, with respect to the chromosome carrying the gene normal.

The alternative hypothesis, that of non-disjunction of the chromosomes carrying waltzing in the male, is not tenable in this instance. Non-disjunction of the chromosomes in the male would produce a gamete with the formula *vv* which on fertilizing a normal ovum would give a triploid zygotic formula of *Vvv*. It is easily conceivable that a double dose of the recessive factor might overbalance the dominant and produce a recessive individual in the $F_1$ generation. As stated above, however, this condition

is not tenable in this instance, for if this $F_1$ waltzer did have the genetic formula of *Vvv* (due to non-disjunction of the two waltzing genes), then in ovogenesis the gametes produced would possess formulae of *V-vv* or *Vv-v* according to the type of reduction.

Now, when the $F_1$ waltzer was mated to a normal male, if her gametes were of the first type, namely, *V* and *vv*, then the possible zygotes would be *VV* and *Vvv*. The last, being similar to the supposed formula of the $F_1$ waltzer, would be a waltzer. But no waltzers were obtained when the $F_1$ waltzer was mated to a normal. Again, if the $F_1$ waltzer were mated to a waltzer and her gametes *V* and *vv* were fertilized by *v* and *v*, zygotes of formulae *Vv* and *vvv* would be produced. At least, the *Vv* combination would be expected to survive and would give a normal (heterozygous) mouse. Here again this condition does not exist, for the $F_1$ waltzer mated to waltzing produces ***only waltzers*** (see table). Hence this hypothesis is untenable.

Similarly, when the other two possibilities are followed out, we find that gametes *Vv* and *v* in matings with a waltzer would give *Vvv* and *vv*, all of which might be expected to be waltzers. Actually, when the $F_1$ is mated to a waltzer all offspring are waltzers. But when the mating is with a normal, zygotes of formulae *VVv* and *Vv* should be produced. Of these, the most likely to survive, would be the *Vv*. These, when mated ***inter se*** should give waltzers in a ratio of 1:3. Actually, they do ***not***, for only normals are produced. If *VVv* zygotes were produced and mated ***inter se***, it is true that they might not produce a waltzer in $F_1$, but would most certainly produce waltzers in $F_2$ or $F_3$. Actually, as stated later, no waltzers are produced in the following two generations at least.

The hypothesis then of non-disjunction of two chromosomes, both carrying the waltzing gene, is not applicable to this case, but non-disjunction of the two chromosomes carrying normal explains all the facts in the case.

It should be borne in mind, then, that the formula of the $F_1$ waltzer is assumed to have been *vo*. If so, she should, in matings with normals, produce zygotes of the formulae *Vv* and *Vo* in equal numbers.

A test of these individuals was made in the case of the third, fourth and last litters produced by the $F_1$ waltzer, these being the litters produced when mated to a normal.

One of the two males (No. 4153) in the third litter, was selected at random to mate with the three female sibs. From this mating, female No. 4151 produced three litters of three, four, and five young respectively, *all* of which offspring were normal. The second female, No. 4152, produced

four litters of six, four, seven, and seven young respectively, *all* normal. The third female, No. 4149, produced a litter of six, four of which were normal and two waltzers. (See table.)

The interesting point with regard to the above is the fact that mated with the same male, the first two females produced no waltzers, whereas the third did produce them. The first two females and the male evidently did not transmit the character waltzing and so must have been of the formula *Vo*. The third can only have been of the formula *Vv*. The male might have been of either formula, so far as the results by the third female are concerned, since in either case the production of waltzers would have been expected. But if he had been of the formula *Vv*, waltzing young should have been produced also by the first two females, whereas they were not produced in totals of 12 and 24 young respectively. It is obvious therefore that he, like the first two females, must have been of the formula *Vo*.

Unfortunately, male No. 4153 was short lived and therefore the young sired by him were limited in number. However, the evidence obtained seems quite conclusive, for statistically, we find that the deviation from 3:1 expectation with the first female is $3 \pm 1.01$ which is 2.97 times the probable error; with the second female the deviation from expectancy is $6 \pm 1.43$ which is 4.19 times the probable error. Combining the two, we have a deviation of $9 \pm 1.75$ which is 5.14 times the probable error. The odds against such an occurrence by chance alone are one to 1,350.

As the $F_1$ waltzer was getting old, and it seemed desirable to get some more young with a genetic constitution of *Vo*, her last mating was with a dilute brown, normal male. From this mating, she produced five young, all normal. Out of this litter one female and one male, Nos. 4808 and 4809 respectively, have shown the same genetic constitution, *Vo*, as the one male and two females from a similar mating described above. No. 4808 mated to her brother, No. 4809, has produced to date three litters of six, six, and five each, respectively. All seventeen are normal. The male, 4809, when mated to heterozygous female sibs produced waltzers as would be expected, for $Vo \times Vv$ gives *VV*, *Vv*, *Vo*, *vo*, the last being a simplex waltzer. Furthermore, male No. 4809 was mated once to female No. 4152 and sired a litter of seven, all of which were normal.

Totaling these matings, we have 36 young from the first series, and 17 plus 7 from the second, a total of 60 young. Remembering that these two series are the $F_2$ generation by a cross between a waltzer and a normal mouse, we should expect to obtain twenty-five percent waltzers, and the writer has shown in a previous paper (Gates, 1926), that this ratio is

maintained under normal conditions. Actually, no waltzers are produced in this case, showing a wholly abnormal Mendelian behavior. The expectation out of a population of 60 would be 15 waltzers. Actually, none was obtained, which is a deviation of $15 \pm 2.26$ from expectation, this being 6.68 times the probable error. The odds against such an occurrence by chance alone are over 100,000 to 1.

It is evident, therefore, that females Nos. 4151, 4152 and 4808 and males Nos. 4153 and 4809 were haplonts or simplex individuals with respect to the waltzing allelomorph, with a genetic constitution of *Vo*. All of the other individuals in these three litters have shown themselves by test to be normal heterozygotes with a genetic constitution of *Vv*.

That the above abnormal Mendelian ratios are certainly due to the haploid condition of the individual is further shown in the matings of females 4151 and 4152 first to heterozygous waltzers, and second, to pure Japanese waltzers. As can be seen in the table, when these two females were mated to heterozygous males, Nos. 4154, 4676 and 4677, waltzers were produced. Likewise, when mated to a homozygous Japanese waltzer, walzers were produced. These results are to be expected, for when *Vo* is crossed with *Vv*, four types of zygotes are to be expected, namely, *VV*, *Vv*, *Vo*, and *vo*, the last only being a waltzer; and when *Vo* is crossed with *vv* two types of young are to be expected, *Vv* and *vo*, the latter being waltzers. The observed results are in harmony with these expectations. (See table.)

The young obtained from *inter se* matings of the five haploid individuals have been random brother-sister mated now for three generations further and 35, 20 and 8 young respectively, obtained. *No* waltzers have appeared, lending added proof to the conclusion that the parents were haplonts, with an entire absence of the waltzing gene.

A further question arises with respect to this mating, namely, of what type is the nulliplex individual when *Vo* is mated to *Vo*, or *vo* to *vo*? Does it survive, or is the combination lethal? None of the matings so far give any evidence of its persistence. It is probably lethal, whether derived through normal individuals or waltzers. There is probably no way to detect it except by further breeding. A nulliplex normal individual (if such an animal exists), mated to a waltzer should produce *all* waltzers ($oo \times vv = vo$ = all simplex waltzers). Such an individual has not yet been found.

In an attempt to identify the nulliplex *oo* individual, derived through waltzers, the waltzers obtained by mating the simplex females, *Vo*, to waltzers or heterozygous waltzers were saved and mated *inter se*. These

should, by hypothesis, have the same genetic constitution as the original $F_1$ waltzer, namely, *vo*; for when *Vo* is mated to *vv*, two types of young in equal proportions are to be expected, *Vv* and *vo*. The former will be normal and heterozygous and the latter waltzing and simplex. When two of the *vo* individuals are bred together they should behave as heterozygotes and should produce three types of young in the ratio of 1:2:1 for $vo \times vo =$ *vv*, 2*vo*, *oo*. The results of these matings have been disappointing, as the individuals seem to be almost sterile, over fifty percent completely so.

However, a few individuals have given birth to one and two litters each and then have died. The total number of litters produced to date is eleven, ranging in size from two to five young, per litter, a total of thirty-nine identified young, *all of which were waltzers*. According to expectation, at least nine or ten young from this number should be nulliplex. It is thus apparent that no nulliplex individuals have survived.

## DISCUSSION

The evidence given above indicates that in the production of the $F_1$ waltzer, there was non-disjunction of the pair of chromosomes carrying the allelomorph of waltzing. An alternate possibility is that this gene was lost from the chromosome in question.[1] Since the mother of the $F_1$ waltzer was homozygous for the allelomorph of waltzing, and since her inheritance behavior was perfectly normal in every case except this one, the loss or non-disjunction must have occurred in ovogenesis. There is no indication of anything unusual in the inheritance through the male parent. The case is entirely explainable on the non-disjunction or the loss hypothesis, involving only the gene normal. The waltzing gene apparently behaved in true Mendelian fashion.

The apparently lethal character of the nulliplex individuals lends support to the non-disjunction hypothesis, for it is less likely that the absence or deficiency of a part of the chromosome would produce death than that the entire absence of two whole chromosomes would do so.

There are one or two matters of considerable interest brought out by this case of non-disjunction. One of these is the added proof that morphological and physiological characters are due to a definite gene or factor. In this case, the factor for normal gait must evidently have disappeared in gametogenesis of an ovum. The introduction of the waltzing factor by the male gamete resulted in the production of the waltzing character in an $F_1$ generation. The gene for waltzing showed by its behavior that it

[1] That this latter hypothesis is the correct explanation, has since been proven by Dr. T. S. PAINTER, UNIVERSITY OF TEXAS. Dr. PAINTER's paper will appear in the July number of Genetics.

was unpaired, that is, simplex or haploid. This was clearly shown when the $F_1$ waltzer was mated to a normal and these bred *inter se*. Waltzing, being completely recessive, the mating of a waltzer to a normal would produce normals, but heterozygous for waltzing. However, some of the $F_1$ from such a mating behaved not as heterozygotes but as simplex normals, *Vo*, which did not carry waltzing, and which, when mated together, *did not* produce waltzers.

When the simplex condition is transferred to normal (formula *Vo*), waltzers apparently cannot be produced. The waltzing factor is necessary for the appearance of waltzing.

Of special interest would be the appearance of the nulliplex individual. The results of the matings recorded above would indicate that it certainly would not be a waltzer. On the other hand, an individual with neither gene for normal would be what? The nulliplex individual has not been identified, either when two simplex normal or two simplex waltzers are mated together. The author hopes, however, to introduce the factor of heterozis in some one of the future generations in such a manner as to have two or more highly vigorous and fertile simplex waltzers. These, mated *inter se*, should produce the nulliplex condition, if it is capable of survival.

The lethal nulliplex probably dies *in utero*, for there has been no evidence of normal or abnormal young dying after birth. As shown in the table, there was no depletion of litters in the case of simplex normals. In the case of simplex waltzers, there has been some depletion but not enough to distort the ratio, nor did the young which died before identification show any abnormal characteristics.

Undoubtedly, other cases of non-disjunction have occurred in mammals but have escaped observation, or perhaps have been discarded because of their obvious non-Mendelian behavior. The appearance of a recessive character in an $F_1$ generation would at once arouse suspicion of double mating and would generally result in the discarding of that particular mating. As in this case, however, such exceptional individuals might prove to be of particular interest.

## SUMMARY

In a mating of a pink-eyed, dilute, brown mouse with a Japanese waltzer a waltzing individual appeared in the $F_1$ generation. This individual proved by breeding tests to have the genetic constitution of a simplex or haplont, *vo*, rather than a heterozygote, *Vo*, as would have been expected.

Mated to a normal this $F_1$ waltzer produced simplex normals, *Vo*, which did not carry waltzing and which when mated *inter se* gave only normals.

The waltzing character is certainly due to a definite gene, for simplex normals, *Vo*, derived from the $F_1$ waltzer do not produce waltzers.

Nulliplex individuals are probably lethal. None have been identified so far.

TABLE 1

*Matings of the $F_1$ waltzer and certain of her progeny.*

| INDIVIDUAL | MATED TO MALE | YOUNG | | CHARACTERISTICS OF YOUNG |
|---|---|---|---|---|
| | | Male | Female | |
| $F_1$ waltzer (*v-o*) | Waltzer (*vv*) | 1 | 4 | all waltzers |
| | Japanese Waltzer (*vv*) | 5 | 1 | all waltzers |
| | Normal (*VV*) | 4 | 2 | all normal |
| | Normal (*VV*) | | 2 | all normal |
| | Waltzer bc (*vv*) | 2 | 2 | all waltzers |
| | Waltzer bc (*vv*) | | 6 | all waltzers |
| | Normal (*Vo*) | 3 | 4 | 2 waltzers, 5 normal |
| | Normal (*VV*) | 2 | 3 | all normal |
| 4151 *V-o* | 4153, Normal (*Vo*) | 3 | | all normal |
| | 4153, Normal (*Vo*) | 2 | 2 | all normal |
| | 4153, Normal (*Vo*) | 3 | 2 | all normal |
| | 4154, Normal (*Vv*) | 2 | 2 | 2 waltzers, 2 normal |
| | 4676, Normal (*Vv*) | 3 | 2 | 3 waltzers, 2 normal |
| | Japanese Waltzer (*vv*) | 2 | 4 | 1 waltzer, 5 normal |
| | Japanese Waltzer (*vv*) | 3 | 1 | 2 waltzers, 2 normal |
| 4152 *V-o* | 4153, Normal (*Vo*) | 5 | 1 | all normal |
| | 4153, Normal (*Vo*) | 2 | 2 | all normal |
| | 4153, Normal (*Vo*) | 3 | 4 | all normal |
| | 4153, Normal (*Vo*) | 4 | 3 | all normal |
| | 4676, Normal (*Vv*) | 2 | | 1 waltzer, 1 normal |
| | 4677, Normal (*Vv*) | 2 | 3 | 3 waltzers, 2 normal |
| | Japanese Waltzer, (*vv*) | 2 | 5 | 2 waltzers, 5 normal |
| | Japanese Waltzer, (*vv*) | 3 | 2 | 2 waltzers, 3 normal |
| | 4809, Normal (*Vo*) | 3 | 4 | all normal |
| 4149 *Vv* | 4153, Normal (*Vo*) | 3 | 3 | 2 waltzers, 2 normal |
| | 4154, Normal (*Vv*) | 5 | 1 | 2 waltzers, 4 normal |
| | 4776, Normal (*Vv*) | 4 | 4 | 1 waltzer, 7 normal |
| 4807 *Vv* | 4809, Normal (*Vo*) | 3 | 4 | 1 waltzer, 6 normal |
| | 4809, Normal (*Vo*) | 2 | 2 | 2 waltzers, 2 normal |
| 4808 *Vo* | 4809, Normal (*Vo*) | 3 | 3 | all normal |
| | 4809, Normal (*Vo*) | 4 | 2 | all normal |
| | 4809, Normal (*Vo*) | | 5 | all normal |

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